# Cannabinoids: Occurrence and Medicinal Chemistry

G. Appendino*[,1], G. Chianese[2] and O. Taglialatela-Scafati*[,2]

[1]*Dipartimento di Scienze Chimiche, Alimentari, Farmaceutiche e Farmacologiche, Università del Piemonte Orientale, Viale Ferrucci 33, 28100 Novara, Italy*

[2]*Dipartimento di Chimica delle Sostanze Naturali, Università di Napoli Federico II, Via D. Montesano 49, 80131 Napoli, Italy*

**Abstract:** With an inventory of several hundreds secondary metabolites identified, *Cannabis sativa* L. (hemp) is one of the phytochemically best characterized plant species. The biomedical relevance of hemp undoubtedly underlies the wealth of data on its constituents and their biological activities, and cannabinoids, a class of unique meroterpenoids derived from the alkylation of an olivetol-like alkyl resorcinol with a monoterpene unit, are the most typical constituents of *Cannabis*. In addition to the well-known psychotropic properties of $\Delta^9$-THC, cannabinoids have been reported to show potential in various fields of medicine, with the capacity to address unmet needs like the relief of chemotherapy-derived nausea and anorexia, and symptomatic mitigation of multiple sclerosis. Many of the potential therapeutic uses of cannabinoids are related to the interaction with (at least) two cannabinoid G-protein coupled receptors ($CB_1$ and $CB_{2)}$. However, a number of activities, like the antibacterial or the antitumor properties are non totally dependent or fully independent from the interaction with these proteins. These pharmacological activities are particularly interesting since, in principle, they could be easily dissociated by the unwanted psychotropic effects.

This review aims at giving readers a survey of the more recent advances in both phytochemistry of *C. sativa*, the medicinal chemistry of cannabinoids, and their distribution in plants, highlighting the impact that research in these hot fields could have for modern medicinal chemistry and pharmacology.

**Keywords:** Cannabinoids, *Cannabis sativa* L., Endocannabinoids, Psychotropic effects, Structure-Activity Relationships, Pain treatment, $\Delta^9$-Tetrahydrocannabinol.

## 1. INTRODUCTION

A combination of history, chemistry, pharmacology, toxicology, and deep social impact makes *Cannabis sativa* L. (hemp) a unique plant. *C. sativa* is relatively unique in taxonomic terms, since the genus *Cannabis* has only one species, and belongs to a family (*Cannabaceae)* including only two genera (*Cannabis* and *Humulus*). Various subspecies of *C. sativa* have been identified [1] but they reflect, however, mainly geographical and/or chemotypic variants of a single taxonomic entity rather than distinct species. From the medicinal point of view, two *Cannabis* phenotypes can be identified: 1) a fiber type *Cannabis*, rich of cannabidiol (CBD) and almost devoid of $\Delta^9$-tetrahydrocannabinol ($\Delta^9$-THC) (generally < 0.2% dry weight)], also called hemp and used for textile or seed oil purposes, and 2) the drug type *Cannabis*, the well-known $\Delta^9$-THC-rich psychotropic cannabis, whose flowering tops are known as marijuana and are used to obtain hashish. The recreational use of the $\Delta^9$-THC-rich chemotype of *C. sativa* dates back to about 5000 years ago and constitutes now the most widely utilized illicit narcotic plant in the world. The psychotropic variety of *Cannabis* was, however, totally unknown to the ancient Mediterranean civilizations and became known in Europe only at the times of the Crusades in the XII-XIII centuries. The wealth of pharmacological activities discovered for *C. sativa* secondary metabolites make this plant one of the most thoroughly investigated from both the phytochemical and the pharmacological points of views, and, at the same time, one of the hottest topics in current medicinal chemistry research.

The chemistry of *C. sativa* is extremely complex and includes constituents belonging to the classes of polyketides, terpenoids (a mixture of about 120 mono- and sesquiterpenoids is responsible for the characteristic odour of the plant), modified sugars, alkaloids, flavonoids, stilbenoids, and quinones. The sheer observation that more than 500 compounds have been characterized from *C. sativa* can give an idea of the phytochemical diversity of this plant. The concentration of these compounds depends on several factors including variety, age, growth conditions, harvesting time and storage conditions [2]. Cannabinoids include about 100 (to date) meroterpenoids (prenylated polyketides) accumulated in tiny epidermal resinous glands and characterized by very specific and potent pharmacological activities, as exemplified by the well-known psychotropic properties of $\Delta^9$-THC. The $\Delta^9$-THC content in cannabis extracts is extremely variable, reaching 15% in some varieties currently available in the illegal market. Recreational cannabis is most often smoked, but this is a relatively inefficient delivery system, since up to 70% of the original $\Delta^9$-THC contents is thermally degraded, with bioavialiablity in the range of 10–27% [3].

In addition to the psychotropic activity of $\Delta^9$-THC, cannabinoids have been reported to show potential in several fields of medicine, with the capacity to address unmet needs like the relief of nausea [4] and anorexia [5] associated to radio- and chemotherapy, and symptomatic mitigation of multiple sclerosis [6]. $\Delta^9$-THC itself has been used to treat glaucoma [7], spasticity from spinal injury [8] or multiple sclerosis, pain and inflammation (see below), and insomnia. Many of the potential therapeutic uses of cannabinoids seem to be related to interaction with the specific cannabinoid receptor system; however, a number of activities, like the antibacterial or the antitumor properties are independent, or non totally dependent, from the interaction with these end-points.

Although there is no shortage of reviews on the biochemical and clinical applications of compounds targeting the cannabinoid receptors (cannabinoids and endocannabinoids) [9-11], less attention has been given to the phytochemistry of *C. sativa* and the medicinal chemistry of cannabinoids, two hot fields that require regular update because of the sheer number of contributions from the primary literature. This review aims at filling this gap, giving readers a survey of the more recent advances in the phytochemistry of *C. sativa*, and highlighting the impact of these discoveries for medicinal chemistry. Cannabinoid-like compounds, as well as cannabigerol (CBG), have been found also in plant unrelated to *C. sativa* and these compounds will be enclosed in this review. Conversely, structurally-unrelated cannabinoid biological analogues of endogenous (endocannabinoids)-, plant (alkamides, polyines)-, and

*Address correspondence to these authors at the Dipartimento di Scienze Chimiche, Alimentari, Farmaceutiche e Farmacologiche, Università del Piemonte Orientale, Viale Ferrucci 33, 28100 Novara, Italy; Tel: +39-0321-375844; Fax: +39 -0321-375821 ; E-mail: appendino@pharm.unipmn.it

Dipartimento di Chimica delle Sostanze Naturali, Università di Napoli Federico II, Via D. Montesano 49, 80131 Napoli, Italy; Tel: +39-081–678509; Fax: +39-081–678552. E-mail: scatagli@unina.it

**0929-8673/11 $58.00+.00** 

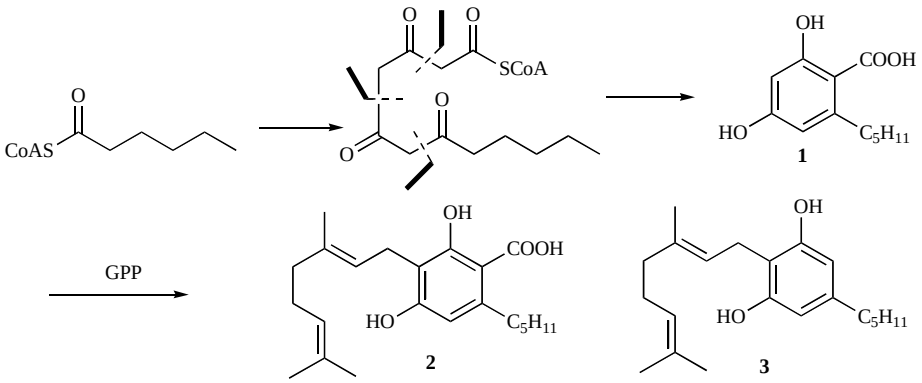



**Fig. (1).** Biogenesis of cannabigerolic acid (**2**) and cannabigerol (**3**).

synthetic origin will not be covered, having all been reviewed recently (see infra).

### 1.1. An ancient Plant

*C. sativa* L. is, arguably, the oldest plant domesticated by man, but its millenary history, crossing most of the human civilization, has always been accompanied by controversy and harsh contrasts. The geographical origin of *Cannabis* is probably Central Asia (Kazakhstan, Western China, and the Russian Far East), where also hop (*Humus lupulus* L.), its only botanical relative, originated [12]. The first known record of its use as a medicine was published in China 5000 years ago during the reign of the Emperor Chen Nung. Another important written description of *Cannabis* use dates back to 2350 B.C. on a stone of the Egyptian Old Kingdom in Memphis, at the end of the Fifth Dynasty [13]. A number of Egyptian papyri reporting *Cannabis* prescriptions (mainly as anti-inflammatory and antimicrobial agent) have been found and dated several centuries B.C. In Islamic countries the resin of *Cannabis* plants was better known as *hashish*, or shadanaj (literally, the royal grain) and widely used for its medical attributes, mainly in the treatment of pain. The name marijuana refers to the flowers and subtending leaves and stalks of mature pistillate of female plants.

As for the Western countries, the first detailed descriptions of the psychotropic variety of hemp dates to the Renaissance. Prosper Alpinus, a Venetian botanist, associated these properties to an Egyptian origin, a view long maintained in the botanical literature. The first impact in Europe occurred after the Napoleon invasion of Egypt. Important members of the French artistic community (Baudelaire, Honore´ de Balzac, Alexandre Dumas, and Gustave Flaubert) under the name "Club des Hashichins" (Hashish Club) met monthly in an old mansion in Paris [14].

Both the fiber and the psychoactive strains became used and investigated but, while opiate alkaloids were isolated early in the 19th century, the identification, isolation, and synthesis of $\Delta^9$-THC was not achieved until 1964 [15]. Investigations on the mechanisms of the psychotropic action of $\Delta^9$-THC yielded to the discovery of the cannabinoid protein receptor family in 1988 and their cloning in early 1990s [16], and the discovery of its endogenous ligand anandamide (the parent compound of the family of endocannabinoids) in 1992 [17]. The last two decades of the twentieth century and the first decade of the present century can be indicated as the "cannabis Renaissance" due to the exceptional blooming of research on *Cannabis* and its metabolites. Although the clinical translation of this research activity is still limited, the recent approval (in Canada, UK and Spain) of Sativex,™ a mixture of natural cannabinoids, constitutes a strong hope for the future.

### 1.2. Cannabis Prohibition

Cultivation and use of psychotropic *Cannabis*, even for medicinal purposes, is illegal in many countries due to the potentially harmful acute and short-lasting effects of this drug. The possible induction of acute psychological side effects (psychosis, depression, and so on) as well as dependency concerns, constitute the strongest arguments of people unfavourable to the therapeutic applications of *Cannabis* products. On the other hand, people seeing with favour a legalization of *Cannabis* use, at least for some medical purposes, deplore the obstacles to the advancement of knowledge caused by the prohibition of its use. In this regard, some scientific journals have strongly supported reconsideration and reclassification of medical *Cannabis*: in 2005, the editor of *Journal of the American Medical Association* [18] pleaded for a reclassification of *Cannabis* in the USA, so that its medical use would be allowed under federal law.

As described in the following paragraphs, the knowledge on the *Cannabis* activity and on its potential has dramatically grown in recent years. The mechanisms of many of the biological activities of *Cannabis* constituents have been elucidated at a receptor level, selective agonists and antagonists at these receptors have been identified and some of them are now undergoing clinical trials. There is the reasonable hope that, in the near future, molecules for which the beneficial effects have been dissociated by the unwanted psychotropic effects will enter the market.

## 2. BIOGENESIS AND CHEMICAL CLASSIFICATION OF CANNABINOIDS

Cannabinoids, a class of mono- to tetracyclic $C_{21}$ (or $C_{22}$) meroterpenoids, are the most important secondary metabolites of *C. sativa*. The plant elaborates these molecules through the assemblage and subsequent modification of two building blocks coming from different biogenetic pathways, namely a $C_{12}$ polyketide unit and a monoterpene unit (geranyl pyrophosphate, GPP), originating from the deoxyxylulose phosphate/methylerythritol phosphate pathway. The first product of the attachment of these two units is cannabigerolic acid, which has been identified as the direct precursor (through subsequent cyclizations and rearrangements) of the most important phytocannabinoid subclasses. A number of reviews have described the secondary metabolism of *C. sativa* in detail [19-21]; in this paragraph we will focus on cannabinoids, highlighting the five most important structural classes and briefly reporting and commenting on their biogenetic relationships.

### 2.1. Cannabigerol-type

As shown in Fig. (**1**), the polyketide moiety of cannabinoids originates from *n*-hexanoylCoA through the tri-fold addition of malonyl-derived acetate units, unit, followed by cyclization and aromatization to give olivetolic acid (**1**). Condensation between olivetolic acid and GPP is catalyzed by a specific prenyltransferase identified in the expanding leaves of *C. sativa* [22]. The product of this reaction is cannabigerolic acid (**2**, CBGA), whose decarboxy-

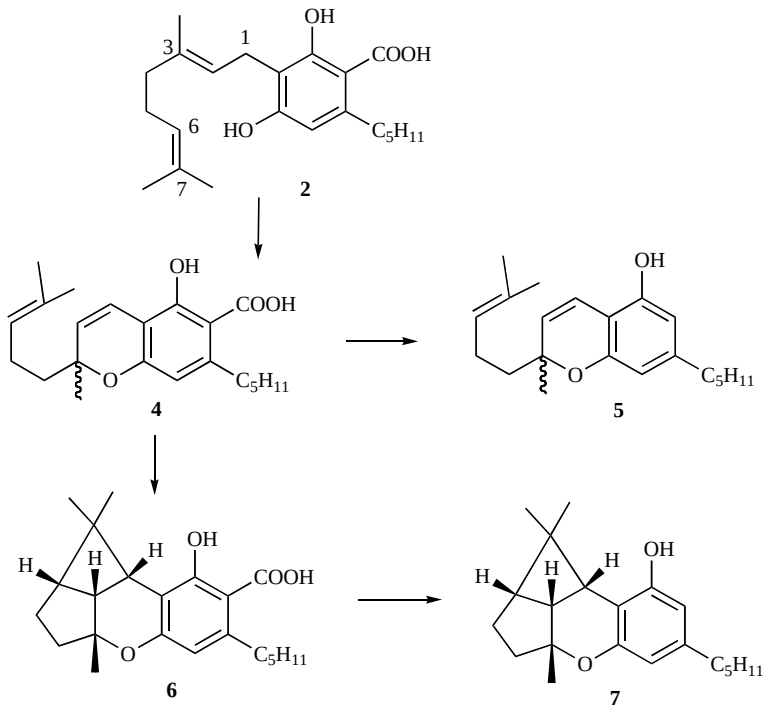



**Fig. (2).** Biogenesis of cannabichromene- and cannabicylol-type compounds.

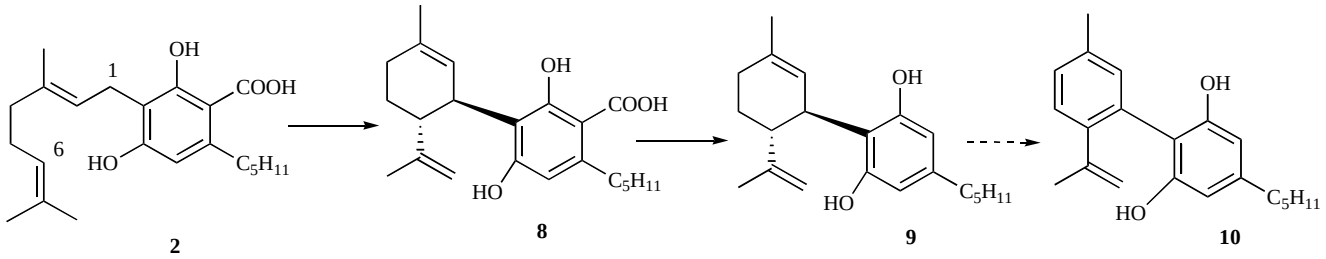



**Fig. (3)**. Biogenesis of cannabidiol (**9**) and cannabinodiol (**10**).

lated analogue is named cannabigerol (**3**, CBG). It is generally accepted that the decarboxylation step for CBGA and all the other cannabinoids is non-enzymatic and occurs spontaneously during either storage or extraction/purification of the compounds.

A series of minor analogues of cannabigerol showing a shorter alkyl chain attached to the phenyl ring have been found, e.g. cannabigerovarin [23] showing a linear $C_3H_7$ chain. These compounds (cannabivarins) could derive from either enzymatic shortening of the CBG pentyl chain or, more likely, from a shorter starter unit (butanoylCoA) for the ketide homologation. Similar shortened-side chain compounds (also with $C_4$ or $C_1$ side chains) have been found in all the other classes of cannabinoids but, for simplicity, they will not be reported in this section of the review.

A CBGA analogue showing a *Z*- double bond in the prenyl unit has been isolated and named cannabinerolic acid [24]. Carmagerol, rac-6',7'-dihydro,6',7'-dihydroxycannabigerol, has been obtained from the Carma variety of *C. sativa* and also identified as a possible mammalian metabolite of cannabigerol [25]. Finally, a number of hydroxylated and oxidized quinone derivatives of cannabigerol have been recently reported from a high-potency $\Delta^9$-THC-rich variety of *C. sativa* [26].

### 2.2. Cannabichromene- and Cannabicyclol-type

The oxidative intramolecular cyclization of CBGA (**2**) affords cannabichromenic acid (CBCA, **4**) and, by decarboxylation, cannabichromene (CBC, **5**). CBC is well represented in psychotropic and fiber–type varieties of *C. sativa* and has been isolated as a racemate [27]. A [2 + 2] intramolecular cycloaddition of CBCA triggers the simultaneous formation of two additional rings (four- and five-membered, respectively) affording the tetracyclic system of cannabicyclolic acid (CBLA, **6**), next turned by decarboxylation into cannabicyclol (CBL, **7**) [28]. It is not clear if the formation of cannabicyclol is the result of natural irradiation on the plant or it is an artifact formed in the crude extract. The photocycloaddition takes place with exquisite diastereoselectivity, and the adduct results from the approach of the terminal double bond from the face *anti* to that of the angular pyrane oxygen. Also cannabicyclol was isolated as a racemate.

### 2.3. Cannabidiol- cannabielsoin- and cannabimovone-type

Cannabidiolic acid (CBDA, **8**) and cannabidiol (CBD, **9**) are the main constituents of the non-psychotropic (fiber-type) varieties of *C. sativa* (Fig. **3**).

These compounds are the result of an oxidative cyclization of CBGA **2,** resulting in the formation of a link between C-1 and C-6 of the prenyl unit. The enzyme catalyzing this stereospecific reaction (cannabidiolic acid synthase) has been isolated and characterized [29]. The aromatized analogue of CBD is named cannabinodiol (**10**) [30], and it is likely an artefact, since its concentration increases with the age of the stored plant.

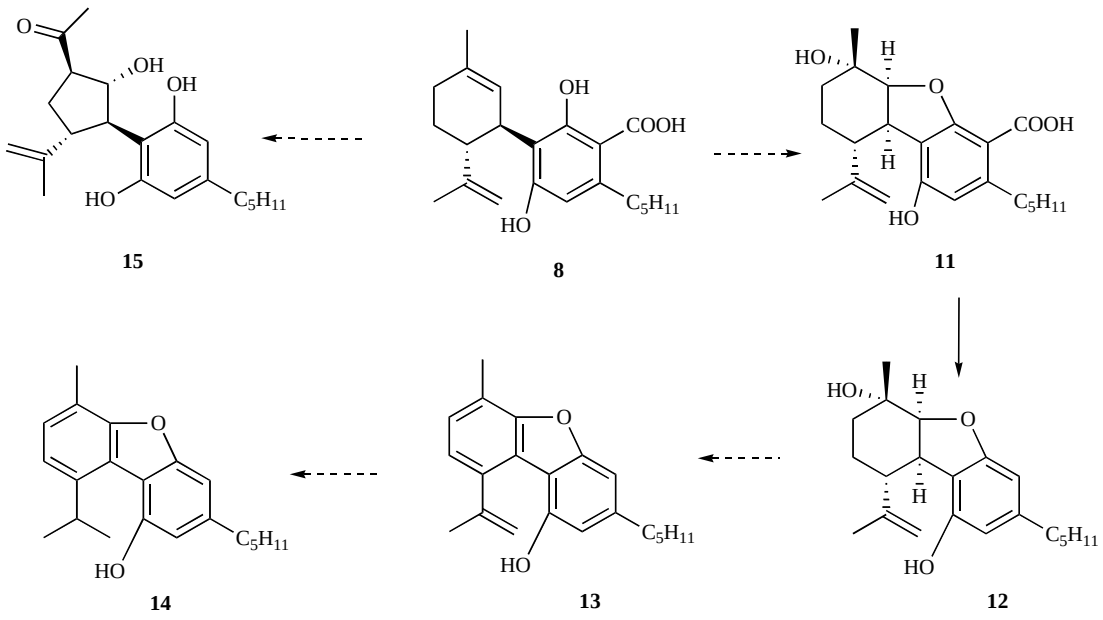



**Fig. (4).** Cannabielsoin- and cannabimovone-type compounds.

Both compounds of the cannabielsoin-cannabifuran type and of the cannabinmovone type likely derive from CBD (Fig. **4**). Although not proven at the biochemical level, and uncharacterized in terms of enzyme(s) involved, these relationships are supported by model reactions that involve intermediates where the trisubstituted double bond of CBD has been epoxidized or dihydroxylated.

Attachment of one of the two phenolic oxygen atoms at the endocyclic double bond of the monoterpene unit generates the dihydrofuran ring of the cannabielsoin (CBE)-type compounds **11** and **12**. These molecules have been both isolated from *C. sativa* [31] but they have also been detected during studies of the mammalian metabolism of CBD [32]. It seems to be present a curious overlapping of cannabinoid oxygenating enzymes between *C. sativa* and mammals, as already suggested by the isolation of carmagerol, another mammalian metabolite of cannabinoids. The aromatized analogue of cannabielsoin, dehydrocannabifuran (**13**) and cannabifuran (CBF, **14**) [33], whose presence has been demonstrated also in the smoke condensate of hashish, show a dibenzofurane structure. Cannabielsoin is the only eponimic cannabinoid, and its name is a tribute to the technician Elsa Boyanova, who isolated it and shortly thereafter passed away.

Cannabimovone (CBM, **15**) was isolated from a non-psychotropic variety of *C. sativa* (Carma) [34]. CBM shows an unprecedented *abeo*-menthane terpenoid structure, seemingly derived from CBD by stereoselective dihydroxylation of the endocyclic double bond, followed by oxidative cleavage of the glycol system and then aldolyzation of the resulting dicarbonyl intermediate. Attempts to reproduce this biogenetic scheme afforded only the cronotized analogue of CBM. Interestingly, this synthetic compound showed a biological profile similar, although less potent, to that of $\Delta^9$-THC, while CBM acted as an analogue of CBD [34].

### 2.4. Tetrahydrocannabinol-type

Tetrahydrocannabinolic acid (THCA, **16**) and $\Delta^9$-tetrahydrocannabinol ($\Delta^9$-THC, **17**) are the main constituents of the psychotropic varieties of *C. sativa*. Although a rearrangement of CBD precursors could seem a reasonable biogenesis for these molecules, the isolation and characterization of the specific FAD-dependent enzyme THCA synthase unambiguously demonstrated that these compounds actually derive from cyclization of CBGA (**2**) through a cationic intermediate with positive charge at C-3 [35]. Attachment of the phenolic oxygen at C-7 of the monoterpene unit and the linkage C-6/C-2 give rise to the tricyclic system of THCA and of its decarboxylated analogue $\Delta^9$-THC (for which the widely employed dibenzopyrane numbering system is indicated in Fig. **5**). These enzymatic subtleties are remarkable on account of the easy cyclization of CBD to $\Delta^9$-THC and its $\Delta^8$-isomer in acidic medium [34].

A number of analogues sharing this structural framework have been found. $\Delta^8$-THCA (**18**) and the decarboxylated **19** show isomerization of the double bond, while the completely aromatized analogues are called cannabinolic acid (CBNA, **20**) and cannabinol (CBN, **21**). These molecules are thought to be artifacts since their concentration in extracts increases during storage, while simultaneously, the concentration of $\Delta^9$-THC decreases. The $\Delta^9$-THC/CBN ratio is used as indication of the age of stored marijuana samples. Hydroxylated CBNA and CBN derivatives have been recently reported [16]. Cannabiripsol (**22**) is the dihydroxylated analogue of $\Delta^9$-THC [36], and it differs from the *trans*- (**23**) and *cis*-cannabitriol (**24**) [37] for a double bond at the junction with the oxygenated ring. Finally dihydroxy-$\Delta^9$-THC (**25**) [38] and cannabitetrol (**26**) [39] differ for the position and /or for the number of hydroxyl groups. Cannabicitran (CBT, **27**) is a cyclized and demethylated analogue of $\Delta^9$-THC [40]. Surprisingly, the configurational aspects of some of these oxygenated analogues of $\Delta^9$-THC, like **25-27**, are still undefined and it is not clear if they were obtained in diastereomerically pure form or as mixtures.

Isotetrahydrocannabinol (**28**) [41] is a minor cannabinoid deriving from a different cyclization mode of the cationic intermediate produced from CBGA (Fig. **6**). The main difference between the two pathways is that, in this case, the phenolic oxygen atom quenches directly the carbocation.

### 2.5. Cannabicoumaronone- and cannabichromanone-type

The carbon skeleton of cannabicoumaronone (**29**) is, in principle, related to that of $\Delta^9$-THC via the oxidative cleavage of its trisubstituted double bond (interestingly, a similar cleavage, on CBD, has been hypothesized for the formation of cannabimovone, CBM) followed by hemiacetalization/dehydration yielding to the

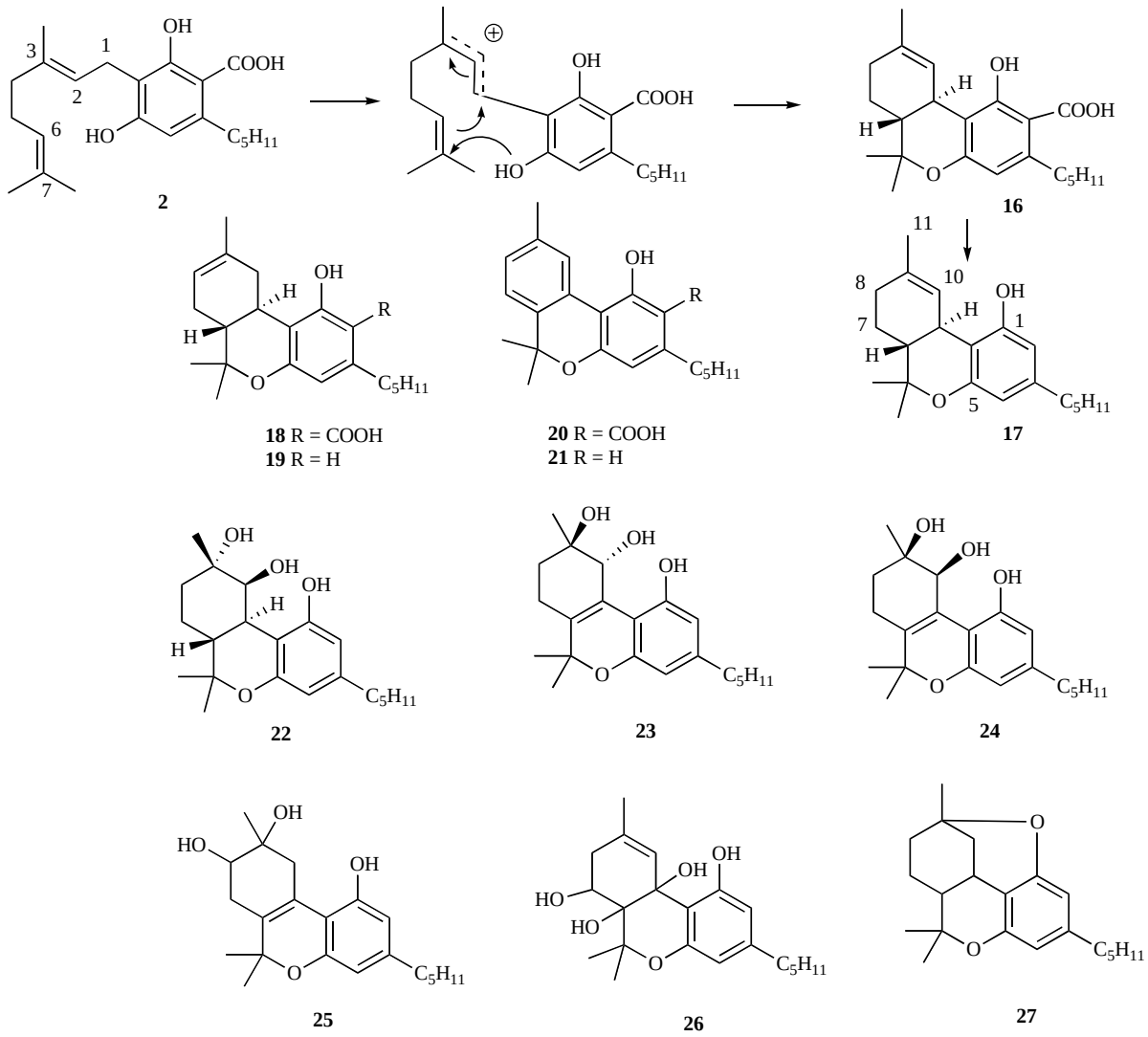



**Fig. (5).** Tetrahydrocannabinol-type compounds.

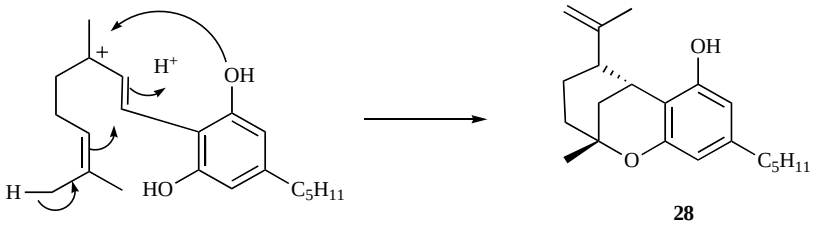



**Fig. (6).** Biogenetic formation of isotetrahydrocannabinol (**28**).

furan ring. A cell suspension culture of *C. sativa* has been demonstrated to be able to convert $\Delta^9$-THC in cannabicoumaronone [42].

Oxidative cleavage of the aldehyde is the likely biogenetic origin of cannabichromanone (**30**), for which a series of derivatives, including the cyclized derivative **31**, have been recently reported [43].

### 2.6. Cannabinoid-like Compounds in Other Plants

The building blocks of cannabinoids, GPP and alkyl resorcinol derivatives, are very common in Nature. For example, olivetol is a common lichen constituent, while limonene, the terpenoid moiety of CBD, is widespread in plants. The question whether cannabinoids are unique to the genus *Cannabis* is therefore interesting, and the occurrence of compounds structurally related to cannabinoids somewhat expected [44].

So far, only two cannabinoids (CBG and its corresponding acid) have been obtained from a non-*Cannabis* source. These compounds are accumulated by a South African *Helichrysum* (*H. umbraculigerum*) [45] and plants from this asteraceous genus (used in traditional medicine for a host of inflammatory and anti-infective conditions) as well as liverworths have also provided compounds related to cannabinoids [45].

*Helychrysum* cannabinoids are prenylated dibenzyls bearing a close relationship with cannabinoids, from which they differ for the replacement of the *n*-pentyl (or *n*-propyl) C-3 side chain with a β-phenylethyl group (e.g. H-CBG, **32**). The two classes of compounds

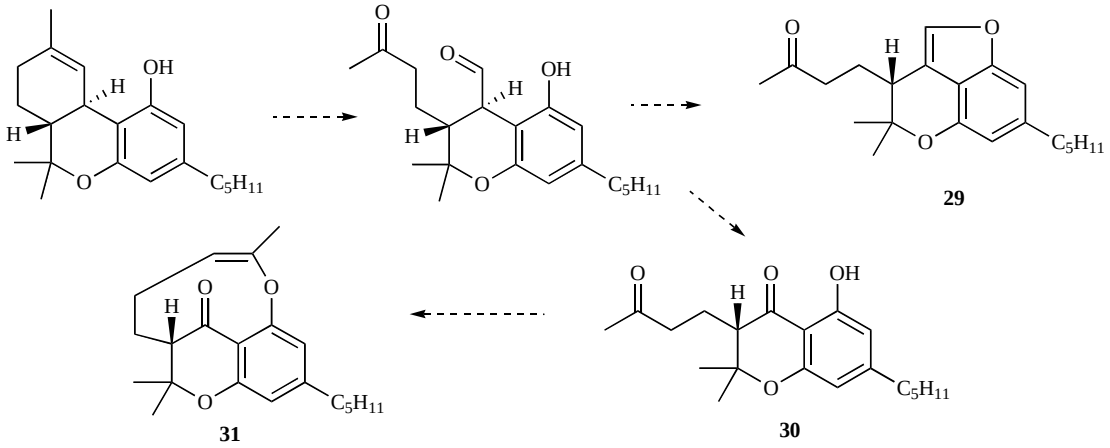



**Fig. (7).** Cannabicoumaronone- and cannabichromanone-type compounds.

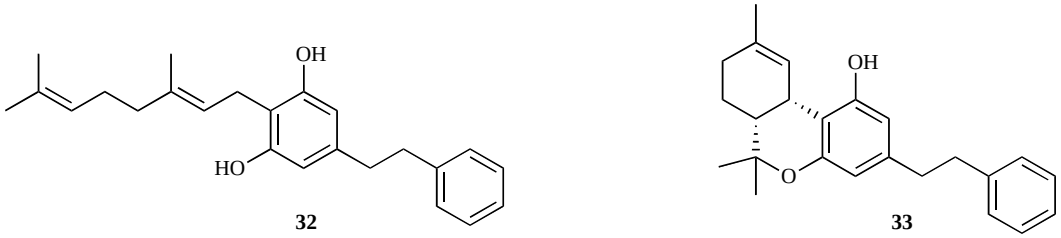



**Fig. (8).** H-CBG (**32**) from *Helichrysum* and **33** from *Radula*.

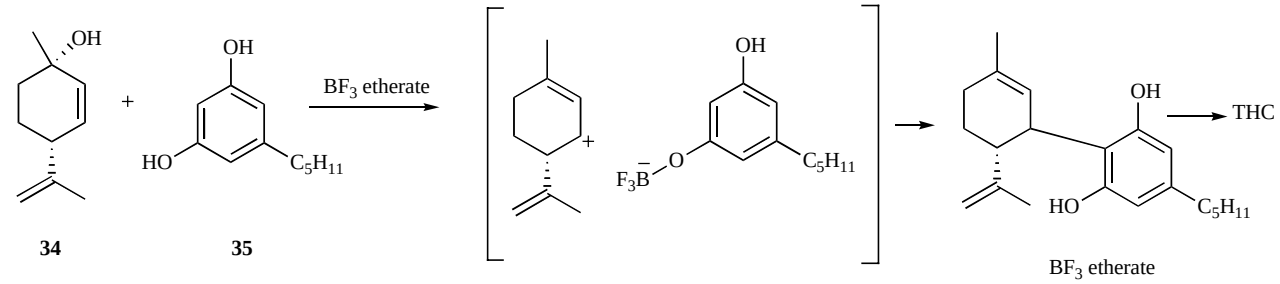



**Fig. (9).** Razdan's synthesis of $\Delta^9$-THC.

are biogenetically related, differing only for the type of ketide starter, which is aliphatic in cannabinoids from hemp, and aromatic in those from *Helichrysum*. Surprisingly, the bioactivity of *Helychrysum* cannabinoids has not yet been investigated, presumably due to their limited availability by isolation or by synthesis. On the basis of the structure-activity relationship of cannabinoids (see below), only modest affinity towards metabotropic cannabinoids receptors ($CB_1$ and $CB_2$) is expected for *Helychrysum* cannabinoids. Some *Helichrysum* cannabinoids have been synthesized (but never investigated biologically) in a seminal work reported by Crombie in the eighties [46].

These prenylated bibenzyl compounds have been found also in tropical liverworts from the genus *Radula*, where they occur with their *ortho*-derivatives (abnormal cannabinoids) [47]. For example, compound **33** strictly resembles $\Delta^9$-THC but no data on its binding to CB receptors are available so far.

Briefly, the research on non-*Cannabis* cannabinoid-like molecules is still in its infancy, but it constitutes a very promising field.

### 2.7. Total Synthesis of Cannabinoids

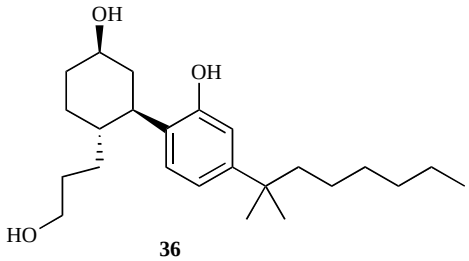
The relatively simple structure of cannabinoids stimulated a great number of different total synthetic approaches, whose detailed account is out of the scope of the present review. Similarly to the biosynthetic pathways, the central step of many of these synthetic routes is the junction of the aromatic portion with the terpene unit. The Razdan's synthesis of $\Delta^9$-THC [48] is reported in Fig. (**9**). This one-step synthesis utilizes the condensation of *p*-menthadienol (**34**) with olivetol (**35**) in the presence of boron trifluoride etherate in DCM. The alternative condensation affording the less stable abnormal CBD has a little impact on the final yields. As obvious from this synthesis, natural CBD from fiber hemp can be employed directly as starting material for the final cyclization, although mixtures of $\Delta^9$ and $\Delta^8$-isomers are generally obtained. Since both isomers have similar psychotropic properties, and since CBD antagonizes the psycothropic properties of $\Delta^9$-THC, it was common practice, before the advent of high-potency street marijuana, to treat the material with lemon juice to cyclise CBD to a mixture of $\Delta^9$-THC and its $\Delta^8$-isomer.

## 3. CANNABINOID RECEPTORS, ENDOCANNABINOIDS AND SYNTHETIC CANNABINOIDS

### 3.1. The Cannabinoid Receptors

Both the structure determination of $\Delta^9$-THC and the discovery of a specific receptor for opioids, two scientific events of the 1960s-1970s, gave a strong impulse to the research on the cannabinoid receptors. The idea that, similarly to opioids, also the psychoactive

constituents from *Cannabis* could act by interaction with a specific receptor located in the CNS, had been long dismissed, also basing on the false assumption that $\Delta^9$-THC and its enantiomer had similar mind-altering activity. Some technical difficulties (e.g. working with highly hydrophobic compounds as cannabinoids) and the lower social and health impact of cannabinoids compared to opioid-derived compounds, also contributed to slow down the research of a cannabinoid receptor. However, one of the most decisive impulse was given by the discovery, in the Pfizer labs, of CP 55,940 (**36**), a synthetic molecule about 20 times more potent than $\Delta^9$-THC [49]. The labelled version of CP 55,940 was used to develop a binding assay for cannabinoid receptors [50], allowing the analysis of their distribution by quantitative autoradiography [51]. High levels of cannabinoid receptors were found in cortex, hippocampus, amygdala, basal ganglia, and in the cerebellum.




**36**

**Fig. (10).** The chemical structure of CP 55,940 (**36**).

Two cannabinoid (CB) receptors have been identified and cloned to date: $CB_1$ and $CB_2$. They have a 40% of homology ($CB_2$ being the smallest one) and both share the heptahelical structure of G-protein coupled receptors [52]. The effect of interaction with cannabinoid receptors is mediated by a cascade of signal transduction pathways including interaction with potassium and calcium channels (for $CB_1$) and several kinases (e.g. MAP kinase). Table **1** summarizes some selected properties of these receptors. The existence of other cannabinoid receptors different from $CB_1$ and $CB_2$ has long been pursued, since a number of cannabinoid-like effects persist in $CB_1/CB_2$ knockout mice [53]. A proposed third cannabinoid receptor is the recently identified GPR55 [54], but its role in the pharmacological actions of $\Delta^9$-THC and in the physiological effects of endogenous cannabinoids are still controversial. GPR55 is activated by lysophosphatidylinositol and is expressed in human and mouse osteoclasts and osteoblasts [55]; it suppresses osteoclast formation but stimulates osteoclast function, exerting a pivotal role in bone physiology and turnover [56].

Many of the psychoactive effects of $\Delta^9$-THC appear to be mediated by $CB_1$ receptors [57], while non-psychoactive cannabinoids (as CBD) have very low affinity both for $CB_1$ and $CB_2$. $\Delta^9$-THC is the phytocannabinoid showing the highest affinity to CB receptors, with a $K_i$ around 40 nM for both $CB_1$ and $CB_2$ (CBN $K_i$ = 150-200 nM, CBD $K_i$ = 3000-4000 nM). $\Delta^8$-THC is almost equipotent to $\Delta^9$-THC [58]. All the cannabinoid acid analogues are free of cannabimimetic CNS activity [59], while, surprisingly, CBD and $\Delta^9$-tetrahydrocannabivarin ($\Delta^9$-THCV) have been found to act as inverse agonist for $CB_1$ [60]. Pertwee *et al.* have reported that $\Delta^9$-THCV is able to antagonize $\Delta^9$-THC in mice *in vivo* [61].

Noteworthy, $CB_2$ receptors are highly expressed in some cells of the immune system [62] and they are believed to play a role in the immune cell function, thus providing a rationale to the immunomodulatory properties of $\Delta^9$-THC [62]. In addition, $CB_2$ receptor is suspected to mediate neuroinflammation, atherosclerosis, and bone remodelling [63]. The localization of both $CB_1$ and $CB_2$ on adipocytes, where their activation appears to stimulate lipogenesis, is particularly interesting and may have a clinical utility [64].

Apart from the metabotropic cannabinoid receptors $CB_1$, $CB_2$ and (possibly) GPR55, cannabinoids also bind to PPAR-gamma and to some thermo-TRPs, a series of ion-channels characterized by an intracellular ligand-binding domain and involved in pain and inflammation [65], (see 4.1). The regulation of ionotropic- and metabotropic proteins targeted by cannabinoids is, undoubtedly, one of the hottest field in cannabinoid research.

Interaction with CB receptors has been unambiguously associated to a number of pharmacological effects, but the most important are: 1) *psychotropic effects* (euphoria). 2) *antiemetic effect*, 3) *analgesic effect*, 4) *immunomodulation* 5) *motor effects* (hypokinesia, ataxia, antispasticity).

Interaction of $\Delta^9$-THC with $CB_1$ receptors on presynaptic nerve terminals in the brain is responsible of the euphoric feelings associated with *Cannabis* use. Some data suggest that this effect could be beneficial in the treatment of depression, but further studies are needed to clarify the role of the cannabinoid system in the neurobiology of this pathology. Other effects of $\Delta^9$-THC in the CNS are ascribable to the presence of cannabinoid receptors in other areas: impairment of cognition and memory (hippocampus)[66]; involuntary movements and partial loss of motor control (basal ganglia and cerebellum) [67]. Since $CB_1$ receptors are not present in the brain region responsible for respiratory and cardiovascular functions, cannabinoid consumption cannot be associated to an increased risk of respiratory or cardiovascular failures, as happens for opiates.

The location of $CB_1$ receptors in cholinergic nerve terminals of the gastrointestinal tract accounts for the THC-induced inhibition of digestive-tract motility [68], whereas the presence of $CB_1$ receptors in the brainstem is responsible of the THC-induced inhibition of emesis [69]. The antiemetic effect of $\Delta^9$-THC has been well established and proposed for treatment of chemotherapy-induced emesis [4], in combination with new generation antiemetic drugs, above all in those patients showing unresponsiveness to the widely used 5-$HT_3$ receptor antagonist ondansetron.

Many studies have reported that $\Delta^9$-THC has a stimulatory effect on appetite and food intake, which can be co-adjuvant in cancer anorexia [5]. In 1992, FDA approved the use of $\Delta^9$-THC to stimulate appetite in AIDS patients suffering from wasting syndrome. This effect could be mediated both by $CB_1$ receptors present in CNS or in nerve terminals and adipocytes.

Most likely, the single most important potential therapeutic effect associated to the interaction with CB receptors is the analgesic effect, due to the role of $CB_1$ receptors in the transmission of nociceptive information in several key tissues. $\Delta^9$-THC has been estimated to be as potent as morphine in blocking nociceptive stimuli in many animal models [70], and, moreover, it can act synergistically with opioid-receptor agonists. Many studies are now aimed at

**Table 1. Some Selected Features of $CB_1$ and $CB_2$ Receptors**

| | **$CB_1$** | **$CB_2$** |
|---|---|---|
| *Type of receptor* | G ($G_i$-$G_o$)-protein coupled | G ($G_i$-$G_o$)-protein coupled |
| *Localization* | Mostly CNS; Adipocytes; Kidney; Lung; Liver | Immune system cells; Spleen; CNS; Osteo-cells; Adipose tissue |
| *Inducibility* | Low inducibility | Highly inducible |

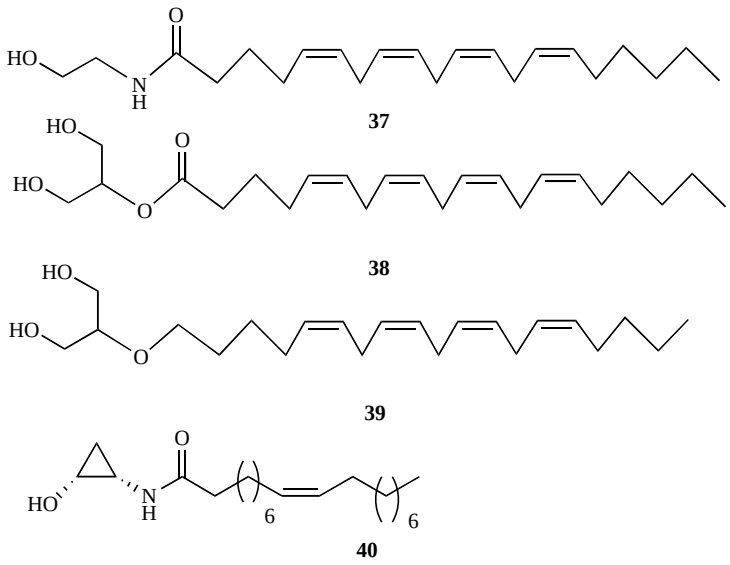



**Fig. (11).** The chemical structures of endocannabinoids **37-39** and the synthetic analogue **40**.

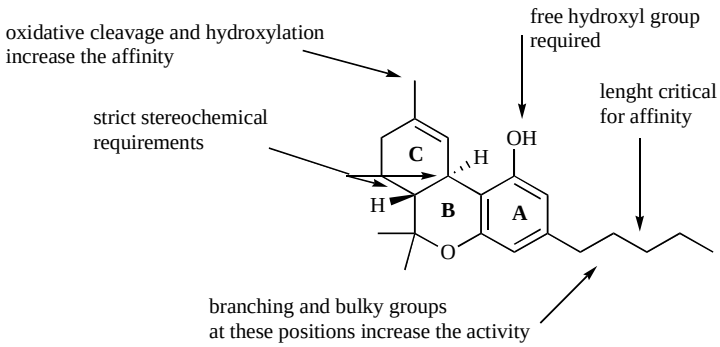



**Fig. (12).** A schematic view of the structure-activity relationships for interaction with the cannabinoid $CB_1$ receptor.

establishing the beneficial effects of a concomitant administration of these drugs.

### 3.2. Endocannabinoids

The presence of cannabinoid receptors implies the existence of endogenous ligands. Mechoulam and Devane identified the first ligand molecule in 1992 [17] and called it anandamide (**37**), from *ananda*, the Sanskrit word to describe "delight, bliss". A number of endogenous $CB_1$ agonist fatty acid amides were then added to the list, while the ester derivative 2-arachidonoyl glycerol (2-AG) (**38**) [71] proved to be more potent than anandamide. Interestingly, virodhamine, an arachidonoyl ethanolamine where the two units are joined by an ester linkage in place of the amide linkage, is an antagonist of $CB_1$ receptor [72] and agonist of $CB_2$ receptor. It is highly produced in peripheral tissues express $CB_2$. Noladin (**39**) and other endocannabinoids have an ether-linkage in place of the ester linkage between the polar head and the apolar tail. Remarkably, all endocannabinoids described to date are either chemical unstable (virodhamine) or rapidly degraded by hydrolytic enzymes *in vivo*.

The endocannabinoids are accompanied by saturated, and mono- or diunsaturated congeners which are $CB_1$ and $CB_2$ receptor-inactive. For example, palmitoylethanolamide has been shown to exhibit antiinflammatory and analgesic activity even though it does not activate central and peripheral cannabinoid receptors [73]. However, palmitoylethanolamide could act stimulating the receptor GPR55 (the postulated new cannabinoid receptor) [74]. In addition, this $CB_1$ or $CB_2$ inactive molecules appear to potentiate the effect of anandamide or arachinoyl glycerol ("entourage effect") [75]. In this regard, we have recently shown [76] that introduction of a methylene lock on the ethanolamide head, thus generating a cyclopropane ring, as in **40**, is able to trigger strong $CB_1$ affinity in oleolyethanolamide.

### 3.3. Structure-activity Relationships of Natural and Synthetic Drugs Targeting the Cannabinoid Receptors (Cannabinoids and Anticannabinoids)

Pharmacological evaluation of the natural compounds and of a number of synthetic molecules allowed the formulation of detailed structure-activity relationships for the interaction with the cannabinoid receptors (see Fig. **12**).

A free hydroxyl group on ring A is required for activity on $CB_1$; presumably due to the formation of hydrogen bonding with the side-chain and the nitrogen atom of Lys12 in transmembrane helix 3 of the CB receptor [77].

The interaction of $\Delta^9$-THC with CB receptors has strict stereochemical requirements and both the enantiomer and the *cis*-diastereomer of $\Delta^9$-THC are inactive or much less active. The same tendency has been observed with synthetic cannabinoids. For example, while the non-classical cannabinoid agonist CP-55,244 (**41**) shows affinity for both $CB_1$ and $CB_2$ in the one-digit nanomolar range, its enantiomer **42** is about 7000 times less active [78]. The structure of compound **41** also highlights the role exerted by the so-called northern aliphatic hydroxyl group (NAH) and the southern aliphatic hydroxyl group (SAH), both of them absent in the structure of classical cannabinoids. Their presence is not crucial for receptor affinity but, most likely, they interact positively (and in a

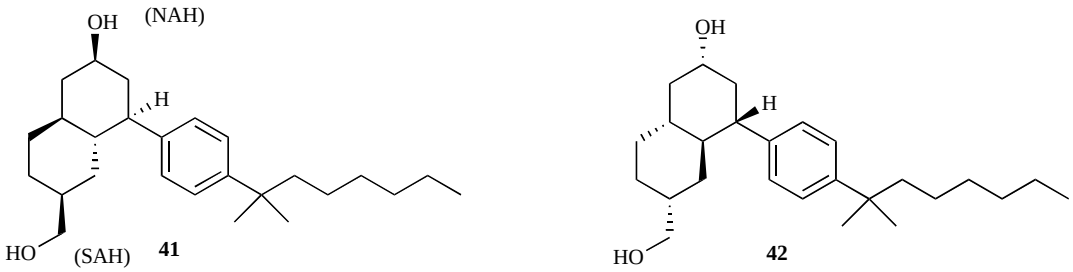



**Fig. (13).** The chemical structure of CP-55,244 (**41**) and its enantiomer **42**.

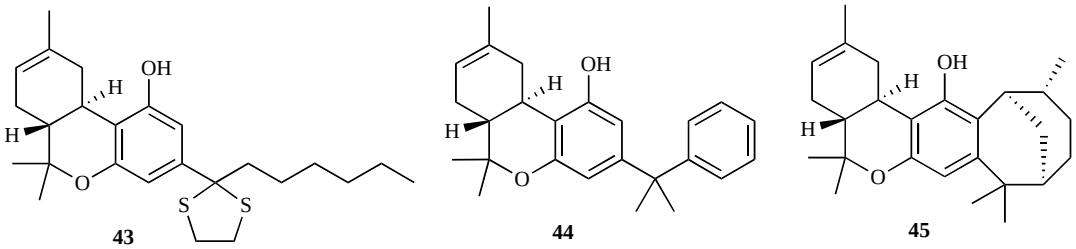



**Fig. (14).** The chemical structures of the modified Δ9-THC derivatives **43-45**.

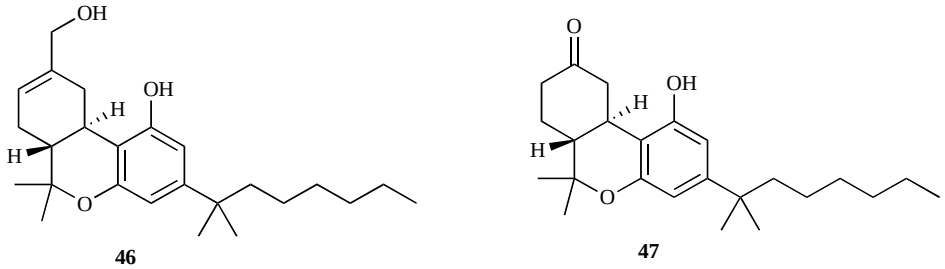



**Fig. (15).** The chemical structures of HU-210 (**46**) and nabilone (**47**).

stereospecific way) with receptorial structures, thus potentiating the activity.

Another critical parameter for CB affinity is the length and the ramification of the pentyl side chain. A decrease in the length of this side chain results in a reduction of potency (THCV is about 75% less potent) and in the appearing of dose-dependent antagonist behaviour [79], while an increase to $C_7$ or $C_8$ results in a systematic increase in affinity. Single or double methyl branching on this side chain also were demonstrated to increase the affinity for $CB_1$ receptor [80]. The most effective ramifications were those at C-1' and C-2' and the *R*-stereoisomers at these stereogenic centers were proved to be more active than the *S*- ones. Likewise, introduction of double or triple bonds at C-1' or C-2' resulted in a greater affinity for both cannabinoid receptors.

The increased affinity for CB receptors of 1',1'-dimethylated alkyl side chains inspired the synthesis of a variety of modified $\Delta^9$- and $\Delta^8$-THC derivatives showing bulky substituents at those positions, some of which are reported in Fig. (**14**) [81]. Thus, compound **43** showed high affinities for both $CB_1$ and $CB_2$ (ab. 0.4 nM), while compound **44** showed a marked selectivity for $CB_2$ receptors. The same behaviour was also shown by the very bulky compound **45** [82].

Modifications at ring C have been mostly concentrated on the allylic methyl group. Hydroxylation of this group on a derivative showing a dimethylated side chain yielded HU210 (**46**), which showed very potent affinities for both $CB_1$ (0.06 nM) and $CB_2$ (0.52 nM) [83].

A similar positive effect is shown by nabilone (**47**), where the methyl group is oxidatively cleaved to give a keto group. Nabilone has been introduced into the market as antiemetic agent for cancer supporting care and, recently, it has been approved in the USA also for the treatment of neuropathic pain [84].

Cannabinoid antagonists could be potentially beneficial in a number of pathological conditions, with obesity (metabolic syndrome) [85] and addiction states (to food, drugs, alcohol, etc.) [86] being probably the most significant and currently investigated examples. Some studies suggest that the effect of cannabinoid anatagonists as antiobesity agent should not only be related to the interaction with CNS $CB_1$ receptors but also to a direct interaction with adipocyte and hepatocyte $CB_1$ receptors [87]. The parent compound of the class of $CB_1$ antagonists/inverse agonist is rimonabant (**48**), a diarylpyrazole derivative with no evident resemblances with the $\Delta^9$-THC structure [88]. Some modifications on the substituents of the pyrazole ring gave SR144528 (**49**), the prototype of the $CB_2$ antagonists, whose application in inflammation and allergies is currently under investigation [89]. Rimonabant was approved for the treatment of obesity and as an aid in the cessation of cigarette smoking, but was later withdrawn from the market because of the severe depression it could induce in sensitive patients.

It should be noted that fifteen years after the discovery of these molecules, as a result of intense medicinal chemistry efforts some significant improvements of their potencies have been achieved but, more importantly, some interesting aspects of their interaction with the receptors have been disclosed [90].

For example, the importance of reduced rotation around the single bond connecting the pyrazole and the monochlorophenyl rings is highlighted by the very potent affinity for $CB_1$ exhibited by NESS0327 (**50**) [91]. Reduction of one of the two double bonds of the pyrazole ring (as in **51**) has been proved to be compatible with high activity [92]. Moreover, an analogue lacking completely the

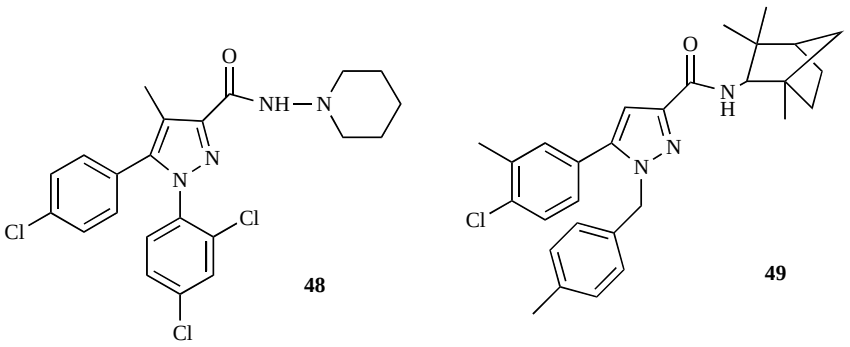



**Fig. (16).** The chemical structures of rimonabant (**48**) and SR144528 (**49**).

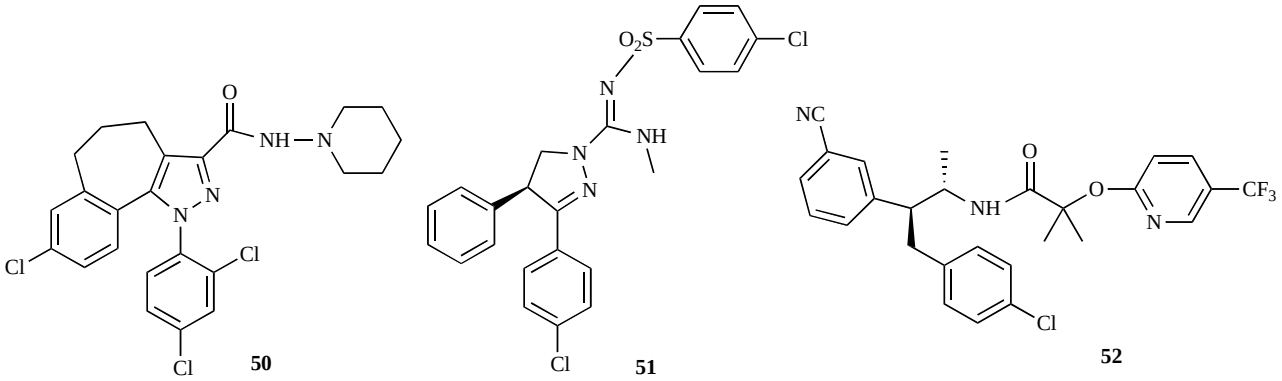



**Fig. (17)**. The chemical structures of the cannabinoid antagonists **50**-**52**.

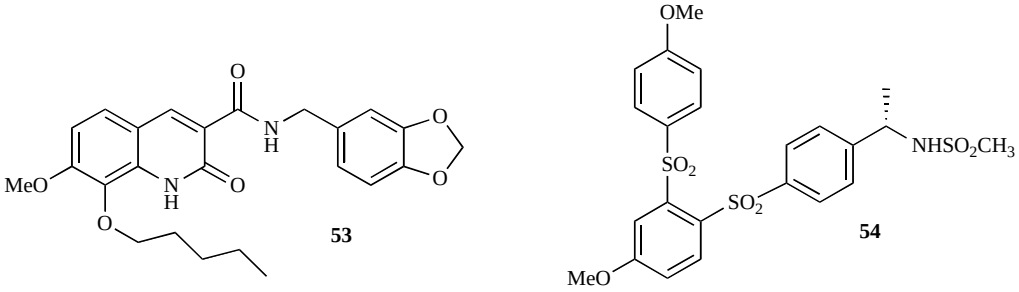



**Fig. (18).** The chemical structures of the $CB_2$ selective antagonists **53** and **54**.

central heterocyclic ring (**52**) also proved to show excellent affinity for $CB_1$ receptor [93].

Also in the case of $CB_2$ selective antagonists a number of structural motifs significantly different from the pyrazole system have been proposed. The most significant examples are the quinoline derivative **53** and the triaryl-bis-sulfone derivative **54** reported in Fig. (**18**).

## 4. OTHER ACTIVITIES, NOT (ENTIRELY) RELATED TO INTERACTION WITH CB RECEPTORS

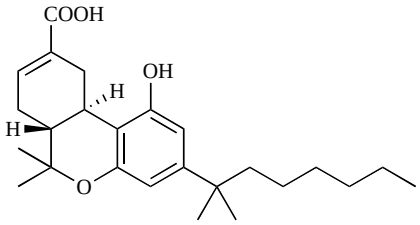
The clinical potential of cannabinoids transcends the effects of their interaction with CB receptors, especially for non-psychotropic compounds [94]. Thus, CBD stimulates PPAR-gamma and the vanilloid receptor type 1 (TRPV1) with a maximum effect similar in efficacy to that of capsaicin [95,96]. Moreover, $\Delta^9$-THC, CBD and CBC have been shown to activate TRPA1 with efficacy comparable with that of mustard oil isothiocyanates, the most potent being CBC ($EC_{50}$ = 60 nM) [97]. Very recently, cannabigerol (CBG) has been demonstrated to behave as a potent $\alpha_2$-adrenoceptor agonist ($EC_{50}$ = 0.2 nM) and to antagonize the 5-$HT_1$A receptor agonist, *R*-(+)-8-hydroxy-2-(di-*n*-propylamino)-tetralin (apparent KB = 51.9 nM) [98]. On the other hand, CBD activates of 5-$HT_1$A receptors with consequent antidepressant-like activity established in mice [99]. Owing to space constraints, we will focus only on the more promising, in terms of clinical translation, of these activities.

### 4.1. Anti-inflammatory Activity

The anti-inflammatory activity of marijuana extracts [100] has been attributed to $\Delta^9$-THC and to non-psychoactive cannabinoids as CBD and CBN. Mouse cells treated with these compounds produced decreased levels of interferons (IFN-α and IFN-β), pro-inflammatory cytokine and chemokine after stimulation with LPS [101], and these effects may be beneficial in some inflammatory/autoimmune diseases. It has been recently proposed that the anti-inflammatory activity of CBD is also mediated by interaction with adenosine signalling [102].

Unlike interaction with CB receptors, anti-inflammatory activity was also shown by carboxy-cannabinoids, as exemplified by ajulemic acid (**55**) a non-ulcerogenic anti-inflammatorial agent capable to suppress 5-lipoxygenase and cyclooxygenase-2 activities [103]. Ajulemic acid has been proposed for treatment of arthritis and for the management of pain and inflammation in multiple sclerosis patients [104]. It should be noticed that ajulemic acid is the dimethylheptyl homolog of the main human metabolite $\Delta^8$-THC.

The name of **55** was apparently based on the initials of the discoverer's grandchildren.




**55**

**Fig. (19).** The chemical structure of ajulemic acid (**55**).

$\Delta^9$-THC, CBD, and CBG have demonstrated a non–CB mediated inhibition of the proliferation of a hyper-proliferating human keratinocyte cell line, supporting a potential role for cannabinoids in the treatment of psoriasis [105].

### 4.2. Neuroprotective Effect

A consistent neuroprotective effect has been evidenced for $\Delta^9$-THC and other cannabinoids, especially for CBD, but it is still unclear whether this effect is mediated by interaction with CB receptors or conversely, if the activity is CBs-independent [106].

An agonist action of CBD on 5-$HT_1$a receptors has been demonstrated, leading to an increased cerebral blood flow and neuroprotective effect [107]. $\Delta^9$-THC-related activation of $CB_1$ receptors on presynaptic terminals of glutamatergic and GABAergic synapses can undoubtedly suppresses the presynaptic release of these neurotransmitters, with potential application for neurodegenerative disorders [108]. However, other studies have demonstrated that the neuroprotection observed with CBD and $\Delta^9$-THC was not affected by cannabinoid-receptor antagonists, and was likely mediated by rather unspecific antioxidant properties [109]. The phenol (THC) or resorcinol (CBD) moieties of cannabinoids are responsible of their antioxidant activity (free-radical scavenging) with a marked preventive effect on hydroperoxide induced oxidative damage. In this context, CBD was found as active as ascorbate or tocopherol [110], a somewhat surprising finding on account of the minor radical-delocalizing properties of this compound.

The non-psychotropic dexanabinol (**56**), enantiomer of HU210 (**46**), and its analogue **57** have been demonstrated to prevent cognitive impairment associated with secondary brain injury induced in animal models [111]. Thus, the cerebroprotective effects of **57** were assessed in rats following head trauma, indicating a significant decrease in oedema and neurological deficits. These molecules act, at least partially, as NMDA non-competitive antagonists suggesting their potential protective value against glutamate excitotoxicity. However, it should also be taken into account that some NMDA antagonists, as MK801, have been demonstrated to induce acute psychosis [112].

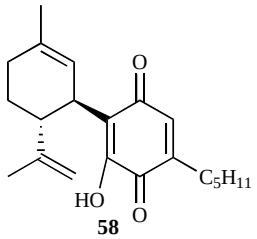
Regardless the mechanism of their neuroprotective action, cannabinoids, and particularly CBD, have been proposed for the treatment of multiple sclerosis and amyotrophic lateral sclerosis, for the improvement in spasticity and pain symptoms. $\Delta^9$-THC has also been proposed for neuroprotection against Parkinson's disease [113], while some CBD analogues have been proposed for neuroprotection in glaucoma to retard the progressive damage of the optic nerve [114].

### 4.3. Antitumor Activity

Similarly to the neuroprotective effect, the antitumor activity of $\Delta^9$-THC and other cannabinoids has not been exclusively associated to the interaction with CB receptors. Indeed, most likely, the tumor death induced by cannabinoids occurs through CB-dependent [115] and/or independent mechanisms basing on the cancer type tested.

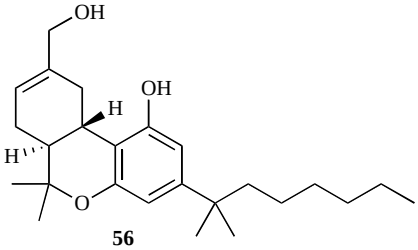
$\Delta^9$-THC was shown to retard the growth of lung adenocarcinoma [116] and to induce apoptosis in C6 glioma cells and prostate cancer cells. The mechanism of these activities is likely CB-receptor independent and seemingly due to activation of mitogen-activated protein (MAP) kinases and interaction with ERK-dependent pathways. Conversely, inhibition of breast cancer growth by $\Delta^9$-THC has been attributed to interaction with $CB_2$ receptors, overexpressed in these tumor cell lines [117].

A renewed interest has also been addressed toward the anticancer potential of CBD, which seems to act through an apoptotic mechanism and activation of caspase-3 [118, 119]. Since CBD can be converted by microsomal enzymes to the hydroxyquinone metabolite HU-331 (**58**), which has been demonstrated to cause apoptosis via the depletion of thiols in splenocytes, it was recently proposed that **58** might be an active metabolite of CBD, potentially contributing to its induction of apoptosis [120]. In addition, a concomitant inhibition of the P-glycoprotein (Pgp)-mediated efflux of doxorubicin has been demonstrated for CBD, a mechanism which could be potentially used to prevent cancer multidrug resistance (MDR) [121]. CBD has also been shown to reverse insensitivity of cancer cells to vinblastine by reducing expression of Pgp [121] and to inhibit BCRP and MRP1, two additional transporters involved in chemoresistance [122-124]. A rational design of phytocannabinoid analogues to better explore the MDR-reversing activity of CBD could be of great interest for drug development.

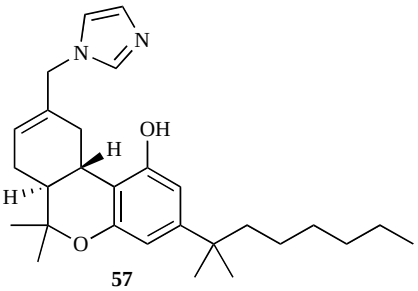



**58**

**Fig. (21).** The chemical structure of the cannabidiol metabolite HU-331 (**58**).




**56**



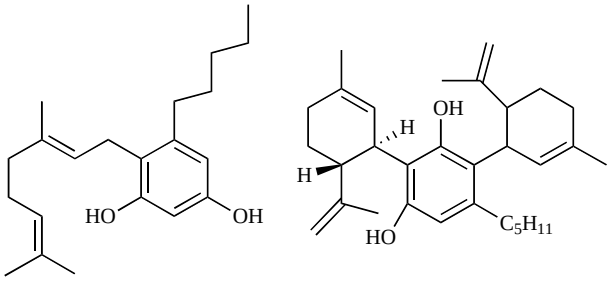

**57**

**Fig. (20).** The chemical structures of the non-psychotropic dexanabinol (**56**) and its analogue **57**.

### 4.4. Antibacterial Activity

*C. sativa* has long been known to contain powerful antibacterial agents and its preparations have been investigated extensively in the 1950s as topical antiseptic agents for the oral cavity and the skin and as antitubercular agents. More recently, data on the antibiotic activity of CBDA, CBC [125], CBG [126], CBD [127], and $\Delta^9$-THC [127] have been obtained, although their comparative evaluation is made difficult by the different bacterial strains used in the various experiments. This activity was not completely surprising since many simple phenols show antimicrobial properties, but both the mechanism of action of cannabinoids and their pharmacophoric regions were not evaluated in these early studies.

Spurred by the diffusion of strains of clinically relevant bacteria that show multidrug-resistance (MDR), like the (in)famous methicillin-resistant *Staphylococcus aureus* (MRSA), and the recently emerged and extremely drug-resistant *Mycobacterium tuberculosis* XDR-TB, the potential of the major cannabinoids to address antibiotic resistance was systematically investigated [128]. All five major cannabinoids (CBD, CBC, CBG, $\Delta^9$-THC and CBN) showed potent activity against a variety of MRSA strains of current clinical relevance (MIC values in the 0.5-2 μg/mL range). Structure-activity studies on the non-psychotropic CBD and CBG showed that the antibacterial activity was remarkably tolerant toward the nature of the prenyl moiety, its relative position compared to the *n*-pentyl moiety (abnormal cannabinoids, e.g. **59**), and toward carboxylation of the resorcinyl moiety (cannabinoid acids). Conversely, methylation and acetylation of the phenolic hydroxyls, esterification of the carboxylic group of cannabinoid acids, and introduction of a second prenyl moiety (e.g. **60**) proved to be detrimental for antibacterial activity. Apparently, the prenyl moiety of cannabinoids serves mainly as a modulator of lipid affinity for the olivetol core, which has per se a only modest antibacterial activity.




**59** **60**

**Fig. (22).** The chemical structures of **59** and **60**.

## 5. CONCLUSIONS

Cannabinoids exemplify the impact that natural products can have on modern medicinal chemistry and pharmacology. Almost one thousand research articles and a host of well-documented reviews have been written in the last three decades on this type of compounds and their biomedical relevance. In retrospect, the clinical translation of this activity seems poor, since, apart from Sativex,™ a mixture of natural cannabinoids (originally approved in Canada, but, more recently, also in Spain and UK, for symptomatic treatment of multiple sclerosis), $\Delta^9$-THC, and its synthetic analogue nabilone, no other drug has emerged from this research. On the other hand, the difficulty inherent to the biological profile of these compounds should also be taken into consideration, since, compared to other neuroactive compounds, cannabinoids are exceedingly pleiotropic in their activity, while their receptors are part of complex neural webs whose manipulation is difficult to predict in terms of *in vivo* effects. Although the debacle of the $CB_1$ inverse agonist rimonabant well exemplifies these difficulties, further investigations in this exciting field are strongly needed.

## LIST OF ABBREVIATIONS

| | | |
|---|---|---|
| CB receptor | = | cannabinoid receptor |
| CBDA | = | cannabidiol acid |
| CBD | = | cannabidiol |
| THCA | = | tetrahydrocannabinolic acid |
| THC | = | tetrahydrocannabinol |
| THCV | = | tetrahydrocannabivarin |
| CBGA | = | cannabigerolic acid |
| CBG | = | cannabigerol |
| CBCA | = | cannabichromenic acid |
| CBC | = | cannabichromene |
| CBLA | = | cannabicyclolic acid |
| CBL | = | cannabicyclol |
| CBE | = | cannabielsoin |
| CBF | = | cannabifuran |
| CBM | = | cannabimovone |
| CBNA | = | cannabinolic acid |
| CBN | = | cannabinol |
| CBT | = | cannabicitran |
| GPP | = | geranyl pyrophosphate |
| 2-AG | = | 2-arachidonoyl glycerol |
| NAH | = | northern aliphatic hydroxyl group |
| SAH | = | southern aliphatic hydroxyl group |
| TRPV1 | = | vanilloid receptor type 1 |
| MAP | = | mitogen-activated protein |
| Pgp | = | P-glycoprotein |
| MDR | = | multidrug resistance |
| MRSA | = | methicillin-resistant *Staphylococcus aureus* |

## REFERENCES

[1] Hillig, K.W. Genetic evidence for separation in *Cannabis* (Cannabaceae). *Genet. Resour. Crop. Evol.*, **2005**, *52*, 161-180.

[2] Keller, A.; Leupin, M.; Mediavilla, V.; Wintermantel, E. Influence of the growth stage of industrial hemp on chemical and physical properties of the fibres. *Ind. Crops Prod.*, **2001**, *13*, 35-48.

[3] Huestis, M.A.; Henningfield, J.E.; Cone, E.J. Blood cannabinoids. I. Absorption of THC and formation of 11-OH-THC and THC-COOH during and after smoking marijuana. *J. Anal. Toxicol.*, **1992**, 16, 276-282.

[4] Garb, S. Cannabinoids in the management of severe nausea and vomiting from cancer chemotherapy. Some additional considerations. *J. Clin. Pharmacol.*, **1981**, *21*, 57S-59S.

[5] Lewis, D.Y.; Brett, R.R. Activity-based anorexia in C57/BL6 mice: Effects of the phytocannabinoid, $\Delta^9$-tetrahydrocannabinol (THC) and the anandamide analogue, OMDM-2. *Eur. Neuropsychopharm.*, **2010**, *20*, 622-631.

[6] de Lago, E.; Gomez-Ruiz, M.; Moreno-Martet, M.; Fernandez-Ruiz, J. Cannabinoids, multiple sclerosis and neuroprotection. *Exp. Rev. Clin. Pharmacol.*, **2009**, *2*, 645-660.

[7] Merritt, J.C.; Perry, D.D.; Russell, D.N.; Jones, B.F. Topical $\Delta^9$-tetrahydrocannabinol and aqueous dynamics in glaucoma. *J. Clin. Pharmacol.*, **1981**, *21*, 467-71.

[8] Maurer, M.; Henn, V.; Dittrich, A.; Hofmann, A. $\Delta^9$-tetrahydrocannabinol shows antispastic and analgesic effects in a single case double-blind trial. *Eur. Arch. Psychiatr. Clin. Neurosci.*, **1990**, *240*, 1-4.

[9] Izzo, A.A.; Borrelli, F.; Capasso, R.; Di Marzo, V.; Mechoulam, R. Non-psychotropic plant cannabinoids: new therapeutic opportunities from an ancient herb. *Trends Pharmacol. Sci.*, **2009**, *30*, 515-527.

[10] Gertsch, J.; Pertwee, R. G.; Di Marzo, V. Phytocannabinoids beyond the Cannabis plant - do they exist? *Br. J. Pharmacol.*, **2010**, *160*, 523-529.

[11] Di Marzo, V.; De Petrocellis, L. Plant, synthetic, and endogenous cannabinoids in medicine. *Ann. Rev. Med.*, **2006**, *57*, 553-574.

[12] Russo, E.B.; Jiang, H.-E.; Li, X.; Sutton, A.; Carboni, A.; del Bianco, F.; Mandolino, G.; Potter, D.J.; Zhao, Y.-X.; Bera, S.; Zhang, Y.-B.; Lu, E.G.; Ferguson, D.K.; Hueber, F.; Zhao, L.C.; Liu, C.-J., Wang, Y.-F.; Li, C.-S. Phytochemical and genetic analyses of ancient cannabis from Central Asia. *J. Exp. Bot.*, **2008**, *59*, 4171-4182.

[13] Russo, E.B. History of Cannabis and Its Preparations in Saga, Science, and Sobriquet. *Chem. Biodiv.*, **2007**, *4*, 1614-1648.

[14] Green J. *Cannabis* Thunder's Mouth Press: New York, **2001**.

[15] Gaoni, Y.; Mechoulam, R. Isolation, structure and partial synthesis of an active constituent of hashish. *J. Am. Chem. Soc*., **1964**, *86*, 1646-1647.

[16] Howlett, A.C.; Johnson, M.R.; Melvin, L.S.; Milne, G.M. Nonclassical cannabinoid analgetics inhibit adenylate cyclase: development of a cannabinoid receptor model. *Mol. Pharmacol.*, **1988**, *33*, 297-302.

[17] Devane, W.A.; Hanus, L.; Breuer, A.; Pertwee, R. G.; Stevenson, L.A.; Griffin, G.; Gibson, D.; Mandelbaum, A.; Etinger, A.; Mechoulam, R. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science*, **1992**, *258*, 1946-1949.

[18] Lawrence, O.; Gostin, J.D. Medical marijuana, American federalism, and the Supreme Court. *J. Am. Med. Assoc.*, **2005**, *294*, 842-844.

[19] Turner, C.E.; Elsohly, M.A.; Boeren, E.G. Constituents of *Cannabis sativa* L. XVII. A review of the natural constituents. *J. Nat. Prod.*, **1980**, *43*, 169-234.

[20] Elsholy, M.A.; Slade, D. Chemical constituents of marijuana: The complex mixture of natural cannabinoids. *Life Sci*., **2005**, *78*, 539-548.

[21] Flores-Sanchez, I.J.; Verpoorte, R. Secondary metabolism in cannabis. *Phytochem. Rev.*, **2008**, *7*, 615-639.

[22] Fellermeier, M.; Zenk, M.H. Prenylation of olivetolate by a hemp transferase yields cannabigerolic acid, the precursor of tetrahydrocannabinol. *FEBS Lett.*, **199**8, *427*, 283-285.

[23] Shoyama, Y.; Hirano, H.; Oda, M.; Somehara, T.; Nishioka, I. Cannabis. IX. Cannabichromevarin and cannabigerovarin, two new propyl homologs of cannabichromene and cannabigerol. *Chem. Pharm. Bull*., **1975**, *23*, 1894-5.

[24] Taura, F.; Morimoto, S.; Shoyama, Y. Cannabinerolic acid, a cannabinoid from *Cannabis sativa*. *Phytochemistry*, **1995**, *39*, 457-8.

[25] Appendino, G.; Giana, A.; Gibbons, S.; Maffei, M.; Gnavi, G.; Grassi, G.; Sterner, O. A. Polar Cannabinoid from *Cannabis sativa* var. Carma. *Nat. Prod. Commun*., **2008**, *3*, 1977-1980.

[26] Radwan M.M.; ElSohly, M.A.; Slade, D.; Ahmed, S.A.; Khan, I.A.; Ross, S.A. Biologically Active cannabinoids from high-potency *Cannabis sativa*. *J. Nat. Prod.*, **2009**, *72*, 906-911.

[27] Gaoni, Y.; Mechoulam, R. Cannabichromene, a new active principle in hashish. *Chem. Commun*., **1966**, 20-21.

[28] Claussen, U.; von Spulak, F.; Korte, F. Haschish XIV. Zur Kenntnis der inhaltsstoffe des haschish. *Tetrahedron*, **1968**, *24*, 1021-1050.

[29] Taura, F.; Morimoto, S.; Shoyama, Y. Purification and characterization of cannabidiolic-acid synthase from *Cannabis sativa L*. Biochemical analysis of a novel enzyme that catalyzes the oxidocyclization of cannabigerolic acid to cannabidiolic acid. *J. Biol. Chem.*,**1996**, *271*, 17411-17416.

[30] Lousberg, R.J.J.C.; Bercht, C.A.L.; Van Ooyen, R.; Spronck, H.J.W. Cannabinodiol: conclusive identification and synthesis of a new cannabinoid from *Cannabis sativa*. *Phytochemistry*, **1977**, *16*, 595-7.

[31] Shani, A.; Mechoulam, R. Cannabielsoic acids. Isolation and synthesis by a novel oxidative cyclization. *Tetrahedron*, **1974**, *30*, 2437-46.

[32] Yamamoto, I.; Gohda, H.; Narimatsu, S.; Watanabe, K.; Yoshimura, H. Cannabielsoin as a new metabolite of cannabidiol in mammals. *Pharmacol. Biochem. Behav.*, **1991**, *40*, 541-546.

[33] Friedrich-Fiechtl, J.; Spiteller, G. New cannabinoids. I. *Tetrahedron*, **1975**, *31*, 479-87.

[34] Taglialatela-Scafati, O.; Pagani, A; Scala, F; De Petrocellis, L; Di Marzo, V.; Grassi, G; Appendino, G. Cannabimovone, a cannabinoid with a rearranged terpenoid skeleton from hemp. *Eur. J. Org. Chem.*, **2010**, *11*, 2067-2072.

[35] Taura, F.; Morimoto, S.; Shoyama, Y.; Mechoulam, R. First direct evidence for the mechanism of Δ1-tetrahydrocannabinolic acid biosynthesis. *J. Am. Chem. Soc.*, **1995**, *117*, 9766-9767.

[36] Boeren, E.G.; Elsohly, M.A.; Turner, C.E. Cannabiripsol: A novel *Cannabis* constituent. *Experientia*, **1979**, *35*, 1278-1279.

[37] Chan, W.R.; Magnus, K.E.; Watson, H.A. The structure of cannabitriol. *Experientia*, **1976**, *32*, 283-284.

[38] Elsohly, M.A.; Boeren, E.G.; Turner, C.E. 9,10-Dihydroxy-6a(10a)-tetrahydrocannabinol and 8,9-dihydroxy-6a(10a)-tetrahydrocannabinol: 2 new cannabinoids from *Cannabis sativa* L. *Experientia*, **1978**, *34*, 1127-1128.

[39] Ross, S.A.; ElSohly, M.A.; Sultana, G.N.N.; Mehmedic, Z.; Hossain, C.F.; Chandra, S. Flavonoid glycosides and cannabinoids from the pollen of *Cannabis sativa* L. *Phytochem. Anal*., **2005**, *16*, 45-48.

[40] Bercht, C.A.L.;. Lousberg, R.J.J., Küppers, F.J.E.M.; Salemink, C.A. Cannabicitran: a new naturally occurring tetracyclic diether from Lebanese *Cannabis sativa*. *Phytochemistry*, **1974**, *13*, 619-621.

[41] Gaoni, Y.; Mechoulam, R. Hashish. XIV. Isotetrahydrocannabinols. *Isr. J. Chem.*, **1968**, *6*, 679-690.

[42] Braemer, R.; Paris, M. Biotransformation of cannabinoids by cell suspension culture of *Cannabis sativa* L. *Plant Cell. Rep*., **1987**, *6*, 150-152.

[43] Ahmed, S.A.; Ross, S.A.; Slade, D.; Radwan, M.M.; Khan, I.A.; ElSohly, M.A. Structure determination and absolute configuration of cannabichromanone derivatives from high potency *Cannabis sativa*. *Tetrahedron Lett.*, **2008**, *49*, 6050-6053.

[44] Woelkart, K.; Salo-Ahen, O.M.H.; Bauer, R. CB receptor ligands from plants. *Curr. Top. Med. Chem.*, **2008**, *8*, 173-186.

[45] Bohlmann, F.; Hoffmann, E. Cannabigerol-ahnliche verbindungen aus *Helichrysum umbraculigerum* (Paper in German). *Phytochemistry*, **1979**, *18*, 1371-1374.

[46] Crombie, L.W., Crombie, W.M.L.; Firth, D.F. Synthesis of bibenzyl cannabinoids, hybrids of two biogenetic series found in *Cannabis sativa*. *J. Chem. Soc. Perkin Trans. I*, **1988**, 1263-1270.

[47] Toyota, M.; Shimamura, T.; Hishii, H.; Renner, M.; Braggings, J.; Asakawa, Y. New Bibenzyl Cannabinoid from the New Zealand Liverwort *Radula marginata*. *Chem. Pharm. Bull.*, **2002**, *50*, 1390-1392.

[48] Razdan, R.K.; Dalzell, H.C.; Handrick, G.R. Hashish. **X.** Simple one-step synthesis of (-)-$\Delta^1$-tetrahydrocannabinol (THC) from *p*-mentha-2,8-dien-1-ol and olivetol. *J. Am. Chem. Soc.*, **1974**, *96*, 5860-5865.

[49] Rinaldi-Carmona, M.; Pialot, F.; Congy, C.; Redon, E.; Barth, F.; Bachy, A.; Brelière, J.C.; Soubrié, P.; Le Fur, G. Characterization and distribution of binding sites for [3H]-SR 141716A, a selective brain ($CB_1$) cannabinoid receptor antagonist, in rodent brain. *Life Sci*., **1996**, *58*, 1239-1247.

[50] Devane, W.A.; Dysarz III, F.A.; Johnson, M.R.; Melvin, L.S.; Howlett, A.C. Determination and characterization of a cannabinoid receptor in rat brain *Mol. Pharmacol.*, **1988**, *34*, 605-613.

[51] Herkenham, M.; Lynn, A.B.; Little, M.D.; Johnson, M.R.; Melvin, L.S.; de Costa, B.R.; Rice, K.C. Cannabinoid receptor localization in brain. *Proc. Natl. Acad. Sci. U.S.A.*, **1990**, *87*, 1932-1936.

[52] Xie, X.-Q.; Chen, J.-Z.; Billings, E.M. 3D structural model of the G-protein-coupled cannabinoid $CB_2$ receptor. *Protein Struct. Funct.Genet*., **2003**, *53*, 307-319.

[53] Jarai, Z.; Wagner, J.A.; Varga, K.; Lake, K.D.; Compton, D.R.; Martin, B.R.; Zimmer, A.M.; Bonner, T.I.; Buckley, N.E.; Mezey, E.; Razdan, R.K.; Zimmer, A.; Kunos, G. Cannabinoid-induced mesenteric vasodilation through an endothelial site distinct from $CB_1$ or $CB_2$ receptors. *Proc. Natl. Acad. Sci. U.S.A.*, **1999**, *96*, 14136-14141.

[54] Sawzdargo, M.; Nguyen, T.; Lee, D.K.; Lynch, K.R.; Cheng, R.; Heng, H.H.; George, S. R.; Dowd, B.F. Identification and cloning of three novel human G protein-coupled receptor genes GPR52, GPR53 and GPR55: GPR55 is extensively expressed in human brain. *Brain Res. Mol. Brain Res.*, **1999**, *64*, 193-198.

[55] Whyte, L.S.; Ryberg, E.; Sims, N.A.; Ridge, S.A.; Mackie, K.; Greasley, P.J.; Ross, R.A.; Rogers, M.J. The putative cannabinoid receptor GPR55 affects osteoclast function *in vitro* and bone mass *in vivo*. *Proc. Natl. Acad. Sci. U.S.A.*, **2009**, *106*, 16511-16516.

[56] Whyte, L.S.; Ryberg, E.; Sims, N.A.; Ridge, S.A.; Mackie, K.; Greasley, P.J.; Ross, R.A.; Rogers, M.J. The putative cannabinoid receptor GPR55 affects osteoclast function *in vitro* and bone mass *in vivo*. *Proc. Natl. Acad. Sci. U. S. A.*, **2009**, *106*, 16511-16516.

[57] Cooper, Z.D.; Haney, M. Cannabis reinforcement and dependence: role of the cannabinoid $CB_1$ receptor. *Addiction Biol*., **2008**, *13*, 188-195.

[58] Huffman, J.W. $CB_2$ receptor ligands. *Mini Rev. Med. Chem.*, **2005**, *5*, 641-649.

[59] Burstein, S. The cannabinoids acids: nonpsychoactive derivatives with therapeutic potential. *Pharmacol. Ther.*, **1999**, *82*, 87-96.

[60] Thomas, A.; Baillie, G.L.; Phillips, A.M.; Razdan, R.K.; Ross, R.A.; Pertwee, R.G. Cannabidiol displays unexpectedly high potency as an antagonist of $CB_1$ and $CB_2$ receptor agonists *in vitro*. *Br. J. Pharmacol*., **2007**, *150*, 613-623.

[61] Pertwee, R.G.; Thomas, A.; Stevenson, L.A.; Ross, R.A.; Varvel, S.A.; Lichtman, A.H.; Martin, B.R.; Razdan, R.K. The psychoactive plant cannabinoid, $\Delta^9$-tetrahydrocannabinol, is antagonized by $\Delta^8$- and $\Delta^9$-tetrahydrocannabivarin in mice *in vivo*. *Br. J. Pharmacol*., **2007**, *150*, 586-594.

[62] Galiegue, S.; Mary, S.; Marchand, J.; Dussossoy, D.; Carriere, D.; Carayon, P.; Bouaboula, M.; Shire, D.; Le Fur, G.; Casellas, P. Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. *Eur. J. Biochem.*, **1995**, *232*, 54-61.

[63] Bab, I.; Ofek, O.; Tam, J.; Rehnelt, J.; Zimmer, A. Endocannabinoids and the regulation of bone metabolism. *J. Neuroendocrinol*., **2008**, *20*, 69-74.

[64] Matias, I.; Gonthier, M.P.; Orlando, P.; Martiadis, V.; De Petrocellis, L.; Cervino, C.; Petrosino, S.; Hoareau, L.; Festy, F.; Pasquali, R.; Roche, R.; Maj, M.; Pagotto, U.; Monteleone, P.; Di Marzo, V. Regulation, function, and dysregulation of endocannabinoids in models of adipose and beta-pancreatic cells and in obesity and hyperglicemia. *J. Clin. Endocrinol. Metab*., **2006**, *91*, 3171-3180.

[65] Akopian A.A.; Ruparel, N.B.; Jeske, N.A.; Patwardhan, A.; Hargreaves, K.M. Role of ionotropic cannabinoid receptors in peripheral antinociception and antihyperalgesia. *Trend Pharmacol. Sci*., **2009**, *30*, 79-84.

[66] Zarrindast, M.-R.; Kangarlu-Haghighi, K.; Khalilzadeh, A.; Fazli-Tabaei, S. Influence of intracerebroventricular administration of cannabinergic drugs on morphine state-dependent memory in the step-down passive avoidance test. *Behav. Pharmacol*., **2006**, *17*, 231-237.

[67] Fernandez-Ruiz, J.; Gonzalez, S. Cannabinoid control of motor function at the basal ganglia. *Handb. Exp. Pharmacol*., **2005**, *168*, 479-507.

[68] Coutts, A.A.; Izzo, A.A. The gastrointestinal pharmacology of cannabinoids: an update. *Curr. Opin. Pharmacol*., **2004**, *4*, 572-579.

[69] Di Carlo, G.; Izzo, A.A. Cannabinoids for gastrointestinal diseases: potential therapeutic applications. *Expert Opin. Investig. Drugs*, **2003**, *12*, 39-49.

[70] Mao, J.; Price, D.D.; Lu, J.; Keniston, L.; Mayer, D. J. Two distinctive antinociceptive systems in rats with pathological pain. *Neurosci. Lett.*, **2000**, *280*, 13-16.

[71] Mechoulam, R.; Ben-Shabat, S.; Hanuš, L.; Ligumsky, M.; Kaminski, N.E.; Schatz, A.R.; Gopher, A.; Almog, S.; Martin, B.R.; Compton, D.R.; Pertwee, R.G.; Griffin, G.; Bayewitch, M.; Barg, J.; Vogel, Z. Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem. Pharmacol.*, **1995**, *50*, 83-90.

[72] Porter, A.C.; Sauer, J.-M.; Knierman, M.D.; Becker, G.W.; Berna, M. J.; Bao, J.; Nomikos, G. G.; Carter, P.; Bymaster, F.P.; Leese, A.B.; Felder, C.C. Characterization of a novel endocannabinoid, virodhamine, with antagonist activity at the $CB_1$ receptor. *J. Pharmacol. Exp. Ther.*, **2002**, *301*, 1020-1024.

[73] Costa, B.; Comelli, F.; Bettoni, I.; Colleoni, M.; Giagnoni, G. The endogenous fatty acid amide, palmitoylethanolamide , has anti-allodynic and anti-hyperalgesic effects in a murine model of neuropathic pain: involvement of CB1, TRPV1 and PPAR-gamma receptors and neurotrophic factors. *Pain*, **2008**, *139*, 541-550.

[74] Godlewski, G.; Offertaler, L.; Wagner, J.A.; Kunos, G. Receptors for acylethanolamides- GPR55 and GPR119. *Prostag. Lipid Mediat.*, **2009**, *89*, 105-111.

[75] Garcia, M.C.; Adler-Graschinsky, E.; Celuch, S.M. Enhancement of the hypotensive effects of intrathecally injected endocannabinoids by the entourage compound palmitoylethanolamide. *Eur. J. Pharmacol.*, **2009**, *610*, 75-80.

[76] 0Appendino, G.; Ligresti, A.; Minassi, A.; Cascio, M.G.; Allarà, M.; Taglialatela-Scafati, O.; Pertwee, R.G.; De Petrocellis, L.; Di Marzo, V. Conformationally constrained fatty acid ethanolamides as cannabinoid and vanilloid receptor probes. *J. Med. Chem.*, **2009**, *52*, 3001-3009.

[77] Shim, J.Y.; Welsh, W.J.; Howlett, A.C. Homology model of the $CB_1$ cannabinoid receptor: sites critical for non-classical cannabinoid agonist interaction. *Biopolymers*, **2003**, *71*, 169-189.

[78] Govaerts, S.; Hermans, E.; Lambert, D.M. Comparison of cannabinoid ligands affinities and efficacies in murine tissues and in transfected cells expressing human recombinant cannabinoid receptors. *Eur. J. Pharm. Sci.*, **2004**, *23*, 233-243.

[79] Razdan, R.K. Structure-activity relationships in cannabinoids. *Pharmacol. Rev.*, **1986**, *38*, 75-149.

[80] Huffman, J.W.; Duncan, S.G.; Wiley, J.L.; Martin, B.R. Synthesis and pharmacology of the 1′,2′-dimethylheptyl-$\Delta^8$-THC isomers: exceptionally potent cannabinoids. *Bioorg. Med. Chem. Lett.*, **1997**, *7*, 2799-2804.

[81] Papahatjis, D.P.; Nikas, S.P.; Kourouli, T.; Chari, R.; Xu, W.; Pertwee, R.G.; Makriyannis, A. Pharmacophoric requirements for the cannabinoid side chain. Probing the cannabinoid receptor subsite at C1'. *J. Med. Chem.*, **2003**, *46*, 3221-3229.

[82] Makriyannis, A. Khanolkar, A. Peripheral cannabinoid receptor ($CB_2$) selective ligands. W.O. Patent 01/28329, April 2001.

[83] Glass, M.; Northup, J.K. Agonist selective regulation of G proteins by cannabinoid $CB_1$ and $CB_2$ receptors. *Mol. Pharmacol.*, **1999**, *56*, 1362-1369.

[84] Frank, B.; Serpell, M. G.; Hughes, J.; Matthews, J.N.S.; Kapur, D. Comparison of analgesic effects and patient tolerability of nabilone and dihydrocodeine for chronic neuropathic pain: randomised, crossover, double blind study. *Br. Med. J.*, **2008**, *336*, 199-201.

[85] Jourdan, T.; Djaouti, L.; Demizieux, L.; Gresti, J.; Verges, B.; Degrace, P. $CB_1$ antagonism exerts specific molecular effects on visceral and subcutaneous fat and reverses liver steatosis in diet-induced obese mice. *Diabetes*, **2010**, *59*, 926-934.

[86] Colombo, G.; Orru, A.; Lai, P.; Cabras, C.; Maccioni, P.; Rubio, M.; Gessa, G. L.; Carai, M.A. M. The cannabinoid $CB_1$ receptor antagonist, rimonabant, as a promising pharmacotherapy for alcohol dependence: preclinical evidence. *Mol. Neurobiol.*, **2007**, *36*, 102-112.

[87] Mackie, K. From active ingredients to the discovery of the targets: the cannabinoid receptors. *Chem. Biodiv.*, **2007**, *4*, 1693-1706.

[88] Rumsfeld, J.S; Nallamothu, B.K. The hope and fear of rimonabant. *J. Am. Med. Assoc.*, **2008**, *299*, 1601-1602.

[89] Rinaldi-Carmona, M.; Barth, F.; Millan, J.; Derocq, J.M.; Casellas, P.; Congy, C.; Oustric, D.; Sarran, M.; Bouaboula, M.; Calandra, B.; Portier, M.; Shire, D.; Breliere, J.C.; Fur, G.L. SR 144528, the first potent and selective antagonist of the $CB_2$ cannabinoid receptor. *J. Pharmacol. Exp. Ther.*, **1998**, *284*, 644-650.

[90] Hurst, D.; Umejiego, U.; Lynch, D.; Seltzman, H.; Hyatt, S.; Roche, M.; McAllister, S.; Fleischer, D.; Kapur, A.; Abood, M.; Shi, S.; Jones, J.; Lewis, D.; Reggio, P. Biarylpyrazole inverse agonists at the cannabinoid $CB_1$ receptor: importance of the C-3 carboxamide oxygen/lysine 3.28(192) interaction. *J. Med. Chem.*, **2006**, *49*, 5969-5987.

[91] Ruiu, S.; Pinna, G. A.; Marchese, G.; Mussinu, J.-M.; Saba, P.; Tambaro, S.; Casti, P.; Vargiu, R.; Pani, L. Synthesis and characterization of NESS 0327: a novel putative antagonist of the $CB_1$ cannabinoid receptor. *J. Pharmacol. Exp. Therap.*, **2003**, *306*, 363-370.

[92] Lange, J.H.M.; Coolen, H.K.A.C.; van Stuivenberg, H.H.; Dijksman, J.A.R.; Herremans, A.H.J.; Ronken, E.; Keizer, H.G.; Tipker, K.; McCreary, A.C.; Veerman, W.; Wals, H.C.; Stork, B.; Verveer, P.C.; Den Hartog, A.P.; De Jong, N.M.J.; Adolfs, T.J.P.; Hoogendoorn, J.; Kruse, C.G. Synthesis, biological properties, and molecular modeling investigations of novel 3,4-diarylpyrazolines as potent and selective $CB_1$ cannabinoid receptor antagonists. *J. Med. Chem.*, **2004**, *47*, 627-643.

[93] Lin, L.S.; Lanza Jr., T.J.; Jewell, J.P.; Liu, P.; Shah, S.K.; Qi, H.; Tong, X.; Wang, J.; Xu, S.S.; Fong, T.M.; Shen, C.-P.; Lao, J.; Xiao, J.C.; Shearman, L.P.; Stribling, D.S.; Rosko, K.; Strack, A.; Marsh, D.J.; Feng, Y.; Kumar, S.; Samuel, K.; Yin, W.; Van der Ploeg, L.H.-T.; Goulet, M.T.; Hagmann, W.K. Discovery of N-[(1S,2S)-3-(4-Chlorophenyl)-2-(3-cyanophenyl)-1-methylpropyl]-2-methyl-2-{[5-(trifluoromethyl)pyridin-2-l]oxy}propanamide (MK-0364), a novel, acyclic cannabinoid-1 receptor inverse agonist for the treatment of obesity. *J. Med. Chem.*, **2006**, *49*, 7584-7587.

[94] De Petrocellis L.; Di Marzo V. Non-CB1, non-CB2 receptors for endocannabinoids, plant cannabinoids, and synthetic cannabimimetics: focus on G-protein-coupled receptors and transient receptor potential channels. *J. Neuroimmun. Pharmacol*., **2010**, *5*, 103-121.

[95] Jacobsson, S.O.; Wallin, T.; Fowler, C.J. Inhibition of Rat C6 glioma cell proliferation by endogenous and synthetic cannabinoids. Relative involvement of cannabinoid and vanilloid receptors. *J. Pharmacol. Exper. Ther.*, **2001**, *299*, 951-959.

[96] Bisogno, T.; Hanus, L.; De Petrocellis, L.; Tchilibon, S.; Ponde, S.D.E.; Brandi, I.; Moriello, A.S.; Davis, J.B.; Mechoulam, R.; Di Marzo, V. Molecular targets for cannabidiol and its synthetic analogues: effect on vanilloid VR1 receptors and on the cellular uptake and enzymatic hydrolysis of anandamide. *Br. J. Pharmacol.*, **2001**, *134*, 845-52.

[97] De Petrocellis, L.; Vellani, V.; Schiano-Moriello, A.; Marini, P.; Magherini, P.C.; Orlando, P.; Di Marzo, V. Plant-derived cannabinoids modulate the activity of transient receptor potential channels of ankyrin type-1 and melastatin type-8. *J. Pharmacol. Exp. Ther.*, **2008**, *325*, 1007-1015.

[98] Cascio, M.G.; Gauson, L.A.; Stevenson, L.A.; Ross, R.A.; Pertwee, R.G. Evidence that the plant cannabinoid cannabigerol is a highly potent $_2$-adrenoceptor agonist and moderately potent $5HT_{1A}$ receptor antagonist. *Br. J. Pharmacol*., **2010**, *159*, 129-141.

[99] Zanelati, T.V.; Biojone, C.; Moreira, F.A.; Guimaraes, F.S.; Joca, S.R.L. Antidepressant-like effects of cannabidiol in mice: possible involvement of $5\text{-}HT_{1A}$ receptors**.** *Br. J. Pharmacol*., **2010**, *159*, 122-128.

[100] Sofia, R.D.; Nalepa, J.J.; Vassar, H.B.; Knobloch, L.C. Comparative antiphlogistic activity of $\Delta^9$-tetrahydrocannabinol, hydrocortisone and aspirin in various rat paw oedema models. *Life Sci.*, **1974**, *15*, 251-260.

[101] Kozela, E.; Pietr, M.; Juknat, A.; Rimmerman, N.; Levy, R.; Vogel, Z. Cannabinoids $\Delta^9$-tetrahydrocannabinol and cannabidiol differentially inhibit the lipopolysaccharide-activated NF-κB and interferon-β/STAT proinflammatory pathways in BV-2 microglial cells. *J. Biol. Chem.*, **2010**, *285*, 1616-1626.

[102] Carrier, E.J.; Auchampach, J.A.; Hillard, C.J. Inhibition of an equilibrative nucleoside transporter by cannabidiol: A mechanism of cannabinoid immunosuppression. *Proc. Natl. Acad. Sci. U.S.A.*, **2006**, *103*, 7895-7900.

[103] Burstein, S.H.; Audette, C.A.; Breuer, A.; Devane, W.A.; Colodner, S.; Doyle, S.A.; Mechoulam, R. Synthetic nonpsychotropic cannabinoids with potent antiinflammatory, analgesic, and leukocyte antiadhesion activities. *J. Med. Chem.*, **1992**, *35*, 3135-3141.

[104] Klein, T.W.; Newton, C.A. Therapeutic potential of cannabinoid-based drugs. *Adv. Exp. Med. Biol.*, **2007**, *601*, 395-413.

[105] Wilkinson, J.D.; Williamson, E.M. Cannabinoids inhibit human keratinocyte proliferation through a non-$CB_1$/$CB_2$ mechanism, and have a potential therapeutic value in the treatment of psoriasis. *J. Dermatol. Sci.*, **2007**, *45*, 87-92.

[106] Sun, Y.; Alexander, S.P.H.; Garle, M.J.; Gibson, C.L.; Hewitt, K.; Murphy, S.P.; Kendall, D.A.; Bennett, A.J. Cannabinoid activation of PPARα: a novel neuroprotective mechanism. *Brit. J. Pharmacol*., **2007**, *152*, 734-743.

[107] Russo, E.B.; Burnett, A.; Hall, B.; Parker, K.K. Agonistic properties of cannabidiol at 5-HT1a receptors. *Neurochem. Res*., **2005**, *30*, 1037-43.

[108] Shen, M.; Thayer, S.A. $\Delta^9$-Tetrahydrocannabinol acts as a partial agonist to modulate glutamatergic synaptic transmission between rat hippocampal neurons in culture**.** *Mol. Pharmacol.*, **1999**, *55*, 8-13.

[109] Chen, J.; Lee, C.T.; Errico, S.; Deng, X.; Cadet, J.L.; Freed, W.J. $\Delta^9$-tetrahydrocannabinol against *N*-methyl-D-aspartate-induced AF5 cell death. *Mol. Brain Res.*, **2005**, *134*, 215-225.

[110] Hampson, A.J.; Grimaldi, M.; Axelrod, J.; Wink, D. Cannabidiol and $\Delta^9$-tetrahydrocannabinol are neuroprotective antioxidants. *Proc. Natl. Acad. Sci. U. S. A.*, **1998**, *95*, 8268-8273.

[111] Darlington, C.L. Dexanabinol: a novel cannabinoid with neuroprotective properties. *Drugs*, **2003**, *6*, 976-979.

[112] Manahan-Vaughan, D.; von Haebler, D.; Winter C.; Juckel, C.; Heinemann, U. A single application of MK801 causes symptoms of acute psychosis, deficits in spatial memory, and impairment of synaptic plasticity in rats. *Hippocampus*, **2008**, *12*, 125-134.

[113] Lastres-Becker, I.; Molina-Holgado, F.; Ramos, J.A.; Mechoulam, R.; Fernandez-Ruiz, J. Cannabinoids provide neuroprotection against 6-hydroxydopamine toxicity *in vivo* and *in vitro*: Relevance to Parkinson 's disease. *Neurobiol. Dis.*, **2005**, *19*, 96-107.

[114] Pertwee, R.G. Pharmacological and therapeutic targets for $\Delta^9$-tetrahydrocannabinol and cannabidiol. *Euphytica*, **2004**, *140*, 73-82.

[115] Aguado, T.; Carracedo, A.; Julien, B.; Velasco, G.; Milman, G.; Mechoulam, R.; Alvarez, L.; Guzman, M.; Galve-Roperh, I. Cannabinoids induce glioma stem-like cell differentiation and inhibit gliomagenesis. *J. Biol. Chem*., **2007**, *282*, 6854-6862.

[116] White, A.C.; Munson, J.A.; Munson, A.E.; Carchman, R.A. Effects of $\Delta^9$-tetrahydrocannabinol in Lewis lung adenocarcinoma cells in tissue culture. *J. Natl. Canc. Inst*., **1976**, *56*, 655-8.

[117] Caffarel, M.M.; Sarriò, D.; Palacios, J.; Guzmàn, M.; Sanchez, C. $\Delta^9$-tetrahydrocannabinol inhibits cell cycle progression in human breast cancer cells through Cdc2 regulation. *Cancer Res.*, **2006**, *66*, 6615-6666.

[118] Massi, P.; Vaccani, A.; Bianchessi, S.; Costa, B.; Macchi, P.; Parolaro, D. The non-psychoactive cannabidiol triggers caspase activation and oxidative stress in human glioma cells. *Cell. Mol. Life Sci.*, **2006**, *63*, 2057-2066.

[119] Ligresti, A.; Moriello, A.S.; Starowicz, K.; Matias, I.; Pisanti, S.; De Petrocellis, L.; Laezza, C.; Portella, G.; Bifulco, M.; Di Marzo, V. Antitumor activity of plant cannabinoids with emphasis on the effect of cannabidiol on human breast carcinoma. *J. Pharmacol. Exp. Therap*., **2006**, *318*, 1375-1387.

[120] Wu, H.-Y.; Jan, T.-R. Cannabidiol hydroxyquinone-induced apoptosis of splenocytes is mediated predominantly by thiol depletion. *Toxicol. Lett.*, **2010**, *195*, 68-74.

[121] Holland, M.L.; Panetta, J.A.; Hoskins, J.M.; Bebawy, M.; Roufogalis, B.D.; Allen, J.D.; Arnold, J.C. The effects of cannabinoids on P-glycoprotein transport and expression in multidrug resistant cells. *Biochem. Pharmacol*., **2006**, *71*, 1146-1154.

[122] Zhu, H.-J.; Wang, J.-S.; Markowitz, J.S.; Donovan, J.L.; Gibson, B.B.; Gefroh, H.A.; DeVane, C.L. Characterization of P-glycoprotein inhibition by major cannabinoids from marijuana. *J. Pharm. Exp. Therap*., **2006**, *317*, 850-857.

[123] Holland, M.L.; Lau, D.T.T.; Allen, J.D.; Arnold, J.C. The multidrug transporter ABCG2 (BCRP) is inhibited by plant-derived cannabinoids. *Br. J. Pharmacol.*, **2007**, *152*, 815-824.

[124] Holland, M.L.; Allen, J.D.; Arnold, J.C. Interaction of plant cannabinoids with the multidrug transporter ABCC1 (MRP1). *Eur. J. Pharmacol*., **2008**, *591*, 128-31.

[125] Turner, C.E.; Elsohly, M.A. Biological activity of cannabichromene, its homologs and isomers. *J. Clin. Pharmacol.*, **1981**, *21*, 283S-291S.

[126] Elsohly, H.N.; Turner, C.E.; Clark, A.M.; Elsohly, M.A. Synthesis and antimicrobial activities of certain cannabichromene and cannabigerol related compounds. *J. Pharm. Sci.*, **1982**, *71*, 1319-1323.

[127] Van Klingeren, B.; Ten Ham, M. Antibacterial activity of $\Delta^9$-tetrahydrocannabinol and cannabidiol. *Lab. Chemother. Natl. Inst. Public Health*, **1976**, *42*, 9-12; *Chem. Abstr*. **1976**, *85*, 14622.

[128] Appendino, G.; Gibbons, S.; Giana, A.; Pagani, A.; Grassi, G.; Stavri, M.; Smith, E.; Rahman, M.M. Antibacterial cannabinoids from *Cannabis sativa*: a structure−activity study. *J. Nat. Prod*., **2008**, *71*, 1427-1430.

**Received: November 29, 2010 Revised: February 16, 2011 Accepted: February 17, 2011**