

Pharmacological and Therapeutic Secrets of Plant and Brain (Endo)Cannabinoids

Lumír Ondřej Hanuš

Department of Medicinal Chemistry and Natural Products, School of Pharmacy,
Faculty of Medicine, Hebrew University, Ein Kerem Campus, Jerusalem 91120, Israel

Published online 5 September 2008 in Wiley InterScience (www.interscience.wiley.com).
DOI 10.1002/med.20135



Abstract: Research on the chemistry and pharmacology of cannabinoids and endocannabinoids has reached enormous proportions, with approximately 15,000 articles on *Cannabis sativa L.* and cannabinoids and over 2,000 articles on endocannabinoids. The present review deals with the history of the *Cannabis sativa L.* plant, its uses, constituent compounds and their biogeneses, and similarity to compounds from *Radula* spp. In addition, details of the pharmacology of natural cannabinoids, as well as synthetic agonists and antagonists are presented. Finally, details regarding the pioneering isolation of the endocannabinoid anandamide, as well as the pharmacology and potential therapeutic uses of endocannabinoid congeners are presented. © 2008 Wiley Periodicals, Inc. Med Res Rev, 29, No. 2, 213–271, 2009

Key words: cannabis; cannabinoids; endocannabinoids; pharmacology; receptors

1. INTRODUCTION

Cannabis sativa L. (hemp) and its phytochemical products (hashish, marihuana) are the most widely produced, plant-based, illicit drugs and the illegal drug most frequently used in Europe. The illegal status and widespread use of Cannabis has simultaneously stimulated efforts to identify the psychoactive constituents of Cannabis while making basic and clinical research on the properties of Cannabis difficult. Despite these obstacles, the discovery of endocannabinoids and delineation of their biochemical and physiological actions in multiple organ systems is currently a significant arena of research. Similarly, the medicinal properties of Cannabis products have been recognized for millennia, but their legal or licensed use in medicine remains controversial. The most prominent therapeutic indications for Cannabis-derived galenicals, or related products (e.g., Marinol) in the modern world include: as an analgesic for cancer pain, post-operative pain, and phantom limb pain;

Correspondence to: Dr. Lumír Ondřej Hanuš, Department of Medicinal Chemistry and Natural Products, School of Pharmacy, Faculty of Medicine, Hebrew University, Ein Kerem Campus, Jerusalem 91120, Israel. E-mail: lumir@cc.huji.ac.il

for decreasing intraocular pressure in glaucoma; reducing spasticity, ataxia, and muscle weakness associated with multiple sclerosis, cerebral palsy, and spinal cord injuries; bronchodilatation in asthma, suppressing emesis induced by oncolytics; and appetite stimulation in response to cachexia/anorexia caused by opioids, antivirals, AIDS-related illnesses or terminal cancer. With active research describing the wide variety of the endocannabinoid system and its receptors currently ongoing, additional medicinal applications for the use of synthetic cannabinoid ligands of greater selectivity and reduced side-effect profile than the galenicals will undoubtedly arise in the future.

The first medicinal applications of plant-derived cannabinoids are lost to time. Nonetheless, they provided the impetus for determining the receptors in the body that mediated their effects, and raised the possibility of endogenous ligands for those receptors. The first steps towards identifying these endocannabinoids, broadening the potential for new therapeutic modalities, began on March 24, 1992. Exactly 555 days after numerous extractions of porcine brains, followed by additional isolations and purifications, this analytical chemist held in his hands a fraction containing the first identified endogenous ligand for the central cannabinoid receptor, N-arachidonylethanolamine, later named anandamide. Thin-layer chromatography revealed that this preparation contained only one compound, and gas chromatography indicated it had one tailing peak, which converted to a sharp, symmetrical peak after silylation. The molecular pharmacologist William Devane established the screening assay, using the central cannabinoid (CB₁) receptor, for pharmacologically identifying the compound. The day after isolating the compound to be named anandamide, its affinity (K_i) for the CB₁ receptor was determined to be 39.0 ± 5.0 nM. In this system, the K_i of Δ⁹-THC, one of the psychoactive components of Cannabis, was determined to be 46.0 ± 3.0 nM. On May 13, 2002, spectrometrist Asher Gopher measured the mass spectrum of the un-derivatized material and determined the molecular weight of the parent ion to be 329 (Fig. 5A). The following day, the molecular weight of the silylated compound was found to be 419, a difference of 18 resulting from the dehydration of the sample during analysis (Fig. 5B). Subsequently, we started to solve the structure of this compound and on July 13, the structure was determined by Raphael Mechoulam to be N-arachidonylethanolamine, who named it anandamide, from the Sanskrit for “eternal bliss.” Aviva Breuer synthesized this compound on August 20, 1992, and the spectra of the natural and synthetic compounds were compared, verifying the structural identification.

The isolation and identification of the first endogenous cannabinoid was an important milestone in the field of cannabinoid pharmacology. The way to this point was very long and started more than 100 years ago. A summary of the work that came before and after the identification of anandamide is the subject of this review.

2. THE NATURAL HISTORY OF CANNABIS

The natural origin of *Cannabis sativa* L., or hemp, is most likely in the regions north of Afghanistan, in the Altai mountains of southern Siberia (Russia). To this day, hemp is a common wild plant in Siberia.¹ It is not clear when and where cultivation of hemp began, but it appears most likely that it originated in north-east Asia (north and north-east China and south-eastern Siberia). Regardless of its origins, there is substantial evidence of the use of hemp as both cordage and medicine throughout recorded human history by societies geographically distant from these origins.

Recent discoveries from Southern Moravia in the Czech Republic provide circumstantial evidence of the oldest use of hemp. The inhabitants of the two most famous eastern Gravettian settlements, the upper paleolithic sites of Pavlov and Dolní Věstonice some 29,000 to 22,000 years ago were expert weavers. The Czech archeologist Klíma unearthed clay fragments bearing a series of impressions from a zone that was radiocarbon dated to between 26,980 and 24,870 years ago.^{2,3} Further excavations of these sites by American and Czech scientists revealed the use of plant fibers in the manufacture of textiles, basketry, cordage, and perhaps netting, with the evidence of these

innovations taking the form of negative impressions of fiber-based constructs on fragments of fired and unfired, or very low-fired, clay. The anthropologists Adovasio and Hyland identified seven of the eight types of twining commonly employed for textiles or basketry at these sites.^{4–7} Hyland also discovered impressions of cordage bearing weaver’s knots.⁸ Mason and her colleagues suggest that the fibrous bark of both alder and yew were locally available and that herbaceous flora including milkweed and nettle,⁹ all of which have well-documented ethnographic and prehistoric uses as perishable production media, including weaving fibers, in Europe.^{10,11} According to Adovasio,¹² the impressions were almost certainly created from fabrics woven of fibers from wild plants, such as nettle or wild hemp, that were preserved by accident.¹³ “Because the impressions of the Pavlov and Věstonice fibers are not of the highest resolution, it is presently only possible to specify which plants they *may* represent. In this case, it *seems* that nettle or, more remotely, wild hemp are possible choices whose presence in the area is attested to by pollen. If we had better impressions, it might be possible to specify with greater precision which of these two plant sources or some other plant source might be represented.”¹² It is tempting to assume that this site may have produced the world’s oldest archeological evidence of Cannabis use.

Somewhat better evidence of the use of hemp by man is provided by the 12,000 years old neolithic site at Yuan-shan on the island of Taiwan. Excavated items include coarse, sandy pottery with hempen cord marks covering the surface, and an incised, rod-shaped stone beater used to pound hemp.^{14,15} An actual fragment of hemp cloth was discovered in 1972 in a grave from the late Chou dynasty (1,122–249 BC) in Shansi province (mainland China), which represents the oldest preserved specimen of Cannabis.¹⁶ Evidence for the medicinal use of Cannabis goes back 5,000 years to the emperor Chen Nung (called the father of Chinese agriculture), alleged discoverer of medicinal plants, who taught his people how to cultivate grains as food. He is said to have tasted hundreds of herbs to test their medicinal value, and is believed to be the author of *Shen-nung pen ts’ao ching* (Divine Husbandman’s *Materia Medica*), the earliest extant Chinese pharmacopoeia. According to this pharmacopoeia, ma-fen, the flowers of the female marijuana plant, contains the greatest amount of yin energy. Yin is the receptive female attribute that is, in traditional Chinese philosophy and medicine, dynamically linked with yang, the creative male element. Ma-fen was prescribed in cases of a loss of yin, such as in menstrual fatigue, rheumatism, malaria, beri-beri, constipation, and absent-mindedness. The *Pen Ts’ao* pharmacopoeia warned that eating too many Ma seeds could cause one to see demons, but that, taken over a long period of time, marijuana seeds could enable one to communicate with spirits.¹⁷

The Assyrians, who ruled large parts of the Middle East (3,000–2,000 BC) also left a pharmaceutical legacy, but on hundreds of clay tablets. Cannabis was one of the major drugs of their pharmacopoeia. They named this plant according to its use. Campbell Thompson identified the Sumerian term *a-zal-la* and the Akkadian term *azallû* as Cannabis on the basis of their similarities to the Aramaic and Syriac *’azal*, meaning “to spin.” He also took the Akkadian word *gurgurru* (probably “cable;” root of this word “garârû” means “to roll, twist round”) as another reference to Cannabis because of its similarity to *garganinj*, the Persian word for Cannabis. Building on these similarities, Campbell then identified the Sumerian drug *gân-zi-gùn-nu* (plant which steals away the mind) as hashish.¹⁸ More recently, a letter written around 680 BC by an unknown woman to the mother of the Assyrian king Esarhaddon, mentions a substance called *qu-nu-bu* which may have been Cannabis.¹⁹

Cannabis was also used by the ancient Egyptians as a medicine. The Ebers Papyrus,^{20–23} found in the tomb at Thebes (the 9th year of the reign of Amenhotep I—approximately 1,534 BC), is not the oldest known recorded mention of the medical uses of Cannabis, but it is the oldest known “complete” medical textbook in existence. The text covers 110 large columns (each of 22 lines on the average) in the original roll, which a modern editor has conveniently divided into 877 numbered sections of varying length, and is by far the most lengthy of the medical papyri. The Ebers Papyrus is a collection of approximately 900 prescriptions, interspersed with diagnosis,

symptoms, physiological descriptions of the action of the heart, concluding with the surgical treatment of wounds and sores. This document mentions in hieratic script the use of medicinal Cannabis under the name šmšmt (shm-shm-t).²⁴ There are two formulas mentioning medicinal use of Cannabis. The first is “A remedy (poultice) for a toe-nail (or finger).” Ingredients include honey, ochre, šmšmt (hemp) and three other ingredients [Formula No. 618 (plate # 78, lines 10–11)]. The second is “A remedy to cool the uterus.” Šmšmt (hemp) is pounded in honey and administered to the vagina. This causes a contraction [of the uterus] [Formula No. 821 (plate # 96, lines 7–8)].

In the Indian scripture of the Atharva Veda, the fourth book of the Vedas, the ancient scriptures of the Brahman religion (ca 2,000–1,400 BC), bhang (hemp) was identified as one of the five sacred plants of India.²⁵ Bhang is “a sacred grass” and its use is considered to “preserve one from disease . . . and prolong the years we have to live.” In the Book II, Hymn IV, 5, we can read “May the hemp and may gangida protect me against vishkandha [hostile demon]! The one (gangida) is brought hither from the forest, the other (hemp) from the sap of the furrow.” In Book XI, Hymn VI, 15: “We speak to the five kingdoms of the plants with soma the most excellent among them. The darbha-grass, hemp, and mighty barley: they shall deliver us from calamity!”

The Aryans who settled in Persia came from the same area in central Russia as their relatives who invaded India, so it is hardly surprising that the Persian word bhang is almost identical to the Indian term bhang. The Persian Zend-Avesta is closely related to the Indian Vedas. However, unlike the Vedas, many of the books that were once a part of the Zend-Avesta have disappeared. The Persian prophet Zarathustra (Zoroaster, ca. 1,200 BC), purported author of the Zend-Avesta, was a user of bhang (hemp). In the Zend-Avesta hemp occupies the first place in a list of 10,000 medicinal plants. One of the few surviving books of the Zend-Avesta, called the Vendidad (The Law Against Demons), calls bhang (Cannabis) Zoroaster’s “good narcotic.” In Vendidad, Fargard 15, II, 14(43) one can read: “And the damsel goes to the old woman and applies to her that she may procure her miscarriage; and the old woman brings her some Banga [Banga is bang or mang, a narcotic made from hempseed], or Shaêta, or Ghnâna, or Fraspâta, or some other of the drugs that produce miscarriage and [the man says], “Cause thy fruit to perish!” and she causes her fruit to perish; the sin is on the head of all three, the man, the damsel, and the old woman.”²⁶ In Khorda Avesta (part 2), 16. Den Yasht, 15 one reads: “To whom the holy Hvovi did sacrifice with full knowledge, wishing that the holy Zarathushtra would give her his good narcotic, that she might think according to the law, speak according to the law, and do according to the law.”²⁷ In the Book of Arda Viraf, which describes the dream-journey of a devout Zoroastrian through the next world is bhang (narcotic) mentioned in Part 1., Chapter 2, 24: “And then those *Dasturs* of the religion filled three golden cups with wine and narcotic of Vishtasp.”²⁸ Similarly, the ancient Aryans that settled in India used Cannabis, but in their worship of the deity Shiva. In one of the Tantric Scriptures we find this revealing statement: “Intoxicating drink (containing bhang) is consumed in order to liberate oneself, and that those who do so, in dominating their mental faculties and following the law of Shiva (yoga) - are to be likened to immortals on earth.” [The Mahanirvana Tantra (XI,105–108).]²⁹

Evidence for the use of Cannabis by Northern Mediterranean societies was first ascribed to Pliny the Elder (79 A.D.), the Roman nobleman, historian, scientist and author of *Naturalis Historia*, (Pliny’s Natural History), who wrote, that “The roots boiled in water ease cramped joints, gout too and similar violent pain.”³⁰ Between 430 and 424 BC the Dorian Greek historian Herodotus of Halicarnassus wrote a book titled *The Histories*, later divided into nine volumes named after the Muses. Herodotus describes the tribal customs of the Scythians, nomads inhabiting what is now southern Russia, and relates how the Scythians inhaled hemp vapors to induce insensibility: “74. Now they have hemp growing in their land . . . the hemp is much superior . . . the garments were of flax or of hemp; and he who had not before seen stuff woven of hemp would suppose that the garment was made of flax . . . 75. The Scythians then take the seed of this hemp and creep under the felt coverings, and then they throw the seed upon the stones which have been heated red-hot: and it burns

like incense and produces a vapor so thick that no vapor-bath in Hellas would surpass it: and the Scythians being delighted with the vapor-bath howl like wolves . . . ”³¹

The Greeks themselves utilized Cannabis for medicinal purposes. The physician, pharmacologist and botanist Pedanius Dioscorides (90 A.D.) described Cannabis in the *Materia Medica*. He described *Kannabis emeros* (Cannabis . . . when it is green is good for the pains of the ears) and *Kannabis agria* (The root being sodden, and so laid on hath ye force to assuage inflammations and to dissolve Oedemata, and to disperse ye obdurate matter about ye joints.)^{32,33} The historian Diodorus Siculus (a Sicilian Greek historian who lived from 90 to 21 BC) reported that the women of Thebes of ancient Egypt used Cannabis as a medicine to relieve sorrow and bad humor.³⁴

During the Hellenistic period, Scythians settled in what is today Israel, in a town south of Kinneret lake named Scythopolis (today's Bet Shean). Nearby Jewish population may have known something of their customs. However, the first secure evidence of the Hebrew use of Cannabis was established in 1936 by Sara Benetowa (Sula Benet), a Polish etymologist.³⁵ The word Cannabis was generally thought to be of Scythian origin, but Benet showed that it has a much earlier origin in Semitic languages like Hebrew, and that it appears several times throughout the Old Testament: “. . . Tracing the history of hemp in terms of cultural contacts, the Old Testament must not be overlooked since it provides one of the oldest and most important written source materials. In the original Hebrew text of the Old Testament there are references to hemp, both as incense, which was an integral part of religious celebration, and as an intoxicant.”³⁶ Cannabis as an incense was also used in the temples of Assyria and Babylon “because its aroma was pleasing to the Gods.”³⁷

Both in the original Hebrew text of the Old Testament and in the Aramaic translation, the word “kaneh” or “keneh” is used either alone or linked to the adjective “bosm” in Hebrew and “busma” in Aramaic, meaning aromatic. It is “cana” in Sanskrit, “qunnabu” in Assyrian, “kenab” in Persian, “kannab” in Arabic and “kanbun” in Chaldean. In Exodus 30: 23, God directed Moses to make a holy oil composed of “myrrh, sweet cinnamon, kaneh bosm and kassia.” In many ancient languages, including Hebrew, the root “kan” has a double meaning—both hemp and reed. In many translations of the Bible's original Hebrew, we find “kaneh bosm” variously and erroneously translated as “calamus” and “aromatic reed,” a vague term. Calamus, (*Calamus aromaticus*) is a fragrant marsh plant. The error occurred in the oldest Greek translation of the Hebrew Bible, Septuagint, in the third century BC, where the terms “kaneh, kaneh bosm” were incorrectly translated as “calamus.” And in the many translations that followed, including Martin Luther's, the same error was repeated. In Exodus 30: 23 “kaneh bosm” is translated as “sweet calamus.” In Isaiah 43: 24 “kaneh” is translated as “sweet cane.” Although the word “sweet” appears nowhere in the original. In Jeremiah 6: 20 “kaneh” is translated as “sweet cane.” In Ezekiel 27: 19 “kaneh” is translated as “calamus.” In Song of Songs 4: 14 “kaneh” is translated “calamus”. Another example that “kaneh,” as used by the Hebrews, means hemp, rather than reed, is the religious requirement that the dead be buried in “kaneh” shirts. Centuries later, linen was substituted for hemp.³⁸

In the course of time, the two words “kaneh” and “bosm” were fused into “kanabos” or “kannabus,” as used in the Mishna, the body of traditional Hebrew law. Thus, the Semitic word “kanbosm” and the Scythian word “Cannabis” may have the same meaning.³⁹ In contrast, Rabin⁴⁰ has suggested that “pannagh” was one of the original forms of the word Cannabis. In Sanscrit bhanga and in Persian bang; in Semitic Assyrian qunnabu, Syrian qunnappa, and classical Arabic kunnab; finally becoming Cannabis in Greek.

Evidence for the entry of Cannabis into modern Western society is provided by the writings of the French physician Jacques Joseph Moreau, which remain the most-cited connection between Cannabis and the art community. Moreau first used hashish while traveling through the Middle East in the 1830s. He hypothesized that Cannabis-induced sensations might model the hallucinations and delusions common in psychotic individuals. He had hoped this research might help treatment of the mentally ill. The outspoken hedonist and popular novelist Pierre Jules Theophile Gautier assisted Moreau in this research. He not only participated himself, but recruited members of France's artistic

community including Charles Pierre Baudelaire, Honoré de Balzac, Alexandre Dumas, and Gustave Flaubert, members of the Club Des Hashichins (Hashish Club), and met monthly in an old mansion in Paris around 1835.⁴¹

The single most complete and authoritative work published on the history of the genus *Cannabis* is by Abel.⁴²

3. CANNABIS SATIVA (HEMP) AND ITS TYPICAL CONTENT COMPOUNDS

A. Cannabinoids from *Cannabis sativa* L.

Cannabis sativa L. (hemp) is a dioecious annual flowering plant. Marihuana is the Spanish name for the dried leaves and female flowering tops of the hemp plant. Hashish is a resin which originates on these female flowering tops. Currently, 538 natural compounds were identified from this plant.⁴³ Of these, 108 are identified as cannabinoids, which are C₂₁ compounds uniquely present in *Cannabis sativa* L. There are ten main types of cannabinoids and fourteen different cannabinoid subtypes (Fig. 1).

The first attempt to successfully identify a cannabinoid was achieved by Wood et al.,⁴⁴ who isolated cannabitol (CBN, C₂₁H₂₆O₂) from the exuded resin of Indian hemp (“charas”). Another milestone in identifying the structure of the cannabinoids was made by Cahn, who determined the structure of CBN,⁴⁵ leaving uncertain only the positions of a hydroxyl and a pentyl group. Several years later, Todd’s group and independently Adam’s group elucidated the correct structure of the first natural cannabinoid, CBN.^{46–48} Subsequently, the second Cannabis constituent, cannabidiol (CBD) was isolated, but its structure was only partially elucidated.⁴⁹ Synthetic tetrahydrocannabinol (THC) derivatives were prepared that showed Cannabis-like activity in animal tests. However, they

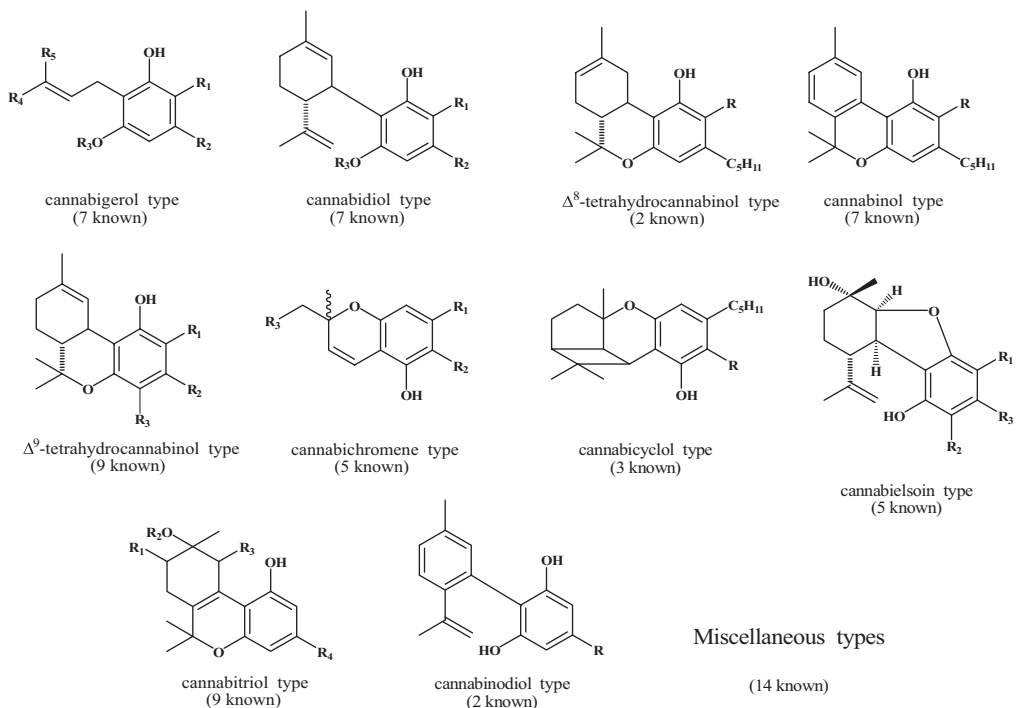


Figure 1. The main types of cannabinoids in *Cannabis sativa* L.

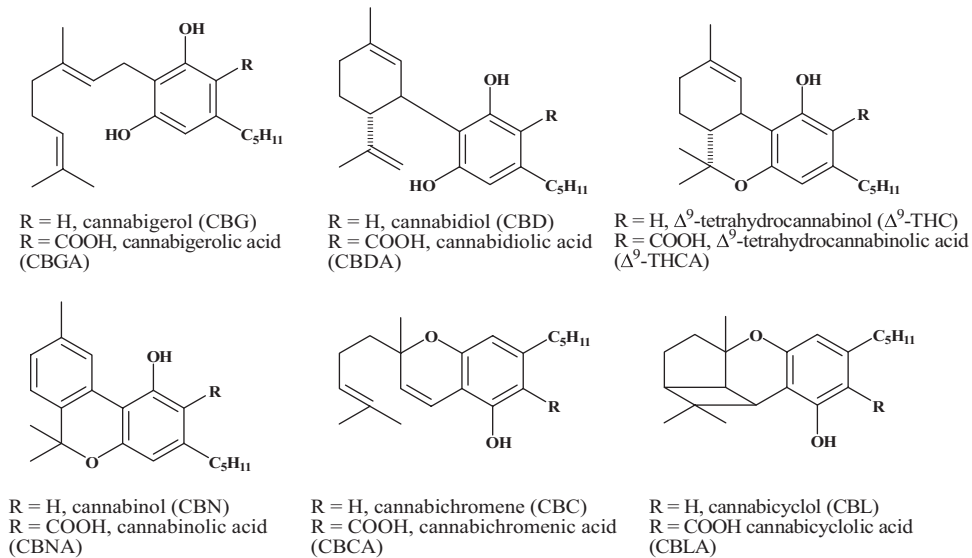


Figure 2. The main cannabinoids in *Cannabis sativa* L.

differed from the active natural product on the basis of their UV spectrum.^{50–53} In a systematic study of the antibacterial substances in hemp, Krejčí and Šantavý found that an extract containing carboxylic acids was effective against *Staphylococcus aureus* and other gram-positive microorganisms. Cannabidiolic acid (CBDA) was isolated from this extract,^{54,55} but the position of the double bond in monoterpene cycle was not determined.

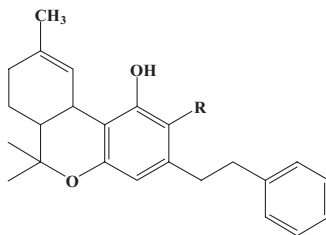
Advances in isolation methods made clarification of Cannabis chemistry possible. In 1963, Mechoulam's group isolated CBD and reported its correct structure and stereochemistry.⁵⁶ A year later they isolated pure tetrahydrocannabinol (Δ^9 -THC), elucidated its structure, obtained a crystalline derivative and achieved a partial synthesis from CBD.⁵⁷ The absolute configuration of CBD and of Δ^9 -THC was established by a shift of the optical rotation value⁵⁸ and by correlation with known terpenoids.⁵⁹ Several years later, a minor, psychotomimetically active constituent, Δ^8 -THC, was isolated from marijuana.⁶⁰ Whether this THC isomer is a natural compound, or an artifact formed during the drying of the plant remains an open question.

Several additional, nonpsychotropic cannabinoids were also identified at that time. The best known are cannabigerol (CBG),⁶¹ cannabichromene (CBC),^{62,63} and cannabicyclol (CBL).⁶⁴ For a better understanding of the biogenesis of these cannabinoids, the isolation and identification of cannabinoid acids was essential. The cannabinolic (CBNA) and cannabigerolic (CBGA) acids were identified,⁶⁵ followed by two Δ^9 -THC acids, A and B,^{66,67} as well as Δ^8 -THC acid^{68,69} and cannabielsoic acid (CBEA).⁷⁰ The decarboxylated product of CBEA, cannabielsoin (CBE), is found in mammals as a metabolite of CBD.⁷¹ Some of the cannabinoid acids have been synthesized.⁷²

It is possible that some of the natural, neutral cannabinoids are artifacts formed through decarboxylation, photochemical cyclization (CBL), oxidation (CBEA) or isomerization (Δ^8 -THCA and Δ^8 -THCA) of other constituents. For the main representative cannabinoids see Figure 2.

B. Cannabinoids from *Radula* Plants

It was presumed that cannabinoids are unique to the Cannabis plant. However, the bibenzyl cannabinoid, perrottetinene, was isolated in 1994 from the small leafy-stem liverwort, *Radula perrottetii*.⁷³ The same compound was found in *Radula laxiramea*.⁷⁴ Later, Toyota et al.⁷⁵ isolated perrottetinene and its acid, perrottetinenic acid in the New Zealand liverwort *Radula marginata*.



R = H, perrottetinene

(1'-benzyl- Δ^9 -tetrahydrocannabiorcol)

R = COOH, perrottetinenic acid

(1'-benzyl- Δ^9 -tetrahydrocannabiorcolic acid A)

C. Biogenesis

Until the mid 1960s the only plant cannabinoid whose structure was fully elucidated was CBN—a constituent which actually may represent an oxidation artifact. Nonetheless, the main cannabinoid structure skeleton was known. Thus, CBD^{76,77} was correctly assumed to be a terpenoid derivative of olivetol, but its exact structure remained unknown. The psychoactive components of Cannabis were assumed to be related tricyclic derivatives. On this basis, Todd suggested that cannabinoids may be formed initially in the plant by condensation of a menthatriene with olivetol. Schultz and Haffner⁷⁸ examined numerous fresh Cannabis plants and found that CBDA predominates over CBD. They assumed that olivetolic acid, not olivetol, is the aromatic species involved in cannabinoid biogenesis. Related biogenetic schemes were put forward by Farmilo et al.⁷⁹ and Grlic,⁸⁰ but a more acceptable biogenetic scheme awaited the isolation of additional cannabinoids and elucidation of their structures. Adams et al.⁸¹ published their data on the conversion of CBD–CBN. Simonsen and Todd⁸² and Todd⁸³ hypothesized that the condensation of menthatriene with olivetol may give rise to a molecule of a type of CBD which can cyclize to THC with the subsequent loss of hydrogen and conversion to CBN.

Based on article and gas chromatography, and color tests of the chemical composition of Cannabis of different origins, a theoretical biogenetic scheme was presented by Farmilo et al.⁷⁹ and Farmilo⁸⁴ Building on Todd's work and using Birch's hypothesis⁸⁵ that acetic acid is the basis for phenol synthesis, they proposed that the first step in cannabinoid biosynthesis was the condensation of hexanoic acid with three molecules of acetic acid to yield cyclohexanedione acid. This intermediate is capable of enolizing to olivetolic acid. If menthadiene (limonene), instead of menthatriene,^{82,83} condensed with olivetolic acid, CBDA could be produced which would decarboxylate to CBD or cyclize to THCA. THCA could decarboxylate to THC with the simultaneous production of CBD by dehydrogenation. Farmilo's hypothesized biogenesis was the first to have considered cannabinoids and their presence in nature as carboxylic acids, particularly THC acid B, years before it was proven to exist. Moreover, the double bond in CBD and THC was placed in the Δ^8 -position, while others put it at Δ^7 . Farmilo's placement of the double bond corresponded to Δ^8 -THC.

Gaoni and Mechoulam⁶¹ stated that CBG probably resulted from the condensation of geranyl pyrophosphate with olivetol, followed by the conversion of CBG to CBD, THC and finally, to CBN.⁶⁵ Two years later,⁸⁶ they described the biogenesis of cannabinoids from geranyl pyrophosphate and olivetol or olivetolic acid (or an open chain precursor of these compounds). This schema was extended⁸⁷ whereby the biogenetic possibilities the production of acidic and neutral cannabinoids were elaborated, with the origination of neutral cannabinoids by decarboxylation of cannabinoid acids. However, the conversion of neutral cannabinoids to cannabinolic acid (CBNA) were demonstrated to be artifacts produced during the harvest and storage of Cannabis.⁶⁷

Razdan⁸⁸ presented a similar biogenetic schema, only with the insertion of an intermediate stage in the formation of CBN from Δ^9 -THC. This intermediate stage is also the origin of the tetrahydrocannabinol ester of CBDA.

De Faubert Maunder⁸⁹ has already put forward his hypothesis of different biogenesis from hemp plants from various geographical localities.

In 1967, Yamauchi et al.⁶⁷ published their study demonstrating neutral cannabinoids and CBN to be artifacts produced during the harvest and storage of Cannabis from cannabinoid acids.

Further clarification of the biosynthetic pathways for cannabinoids applied labeled precursors, such as calcium malonate-[2-¹⁴C], sodium mevalonate-[2-¹⁴C], geraniol-[1-³H], nerol-[1-³H], and CBGA-carboxyl-¹⁴C, CBGA-[U-³H] and CBDA-carboxyl-¹⁴C, directly to leaves, or to the plant through a cotton wick, for a period of 6 days.^{90,91}

Several investigations indicate that there are significant regional differences in the biosynthesis of cannabinoids. For example, a variety of Japanese hemp was found to contain cannabispirene and acetyl cannabispirene, in addition to cannabispirene and cannabispirenone,⁹² with n-propyl cannabinoid acids such as cannabigevarinic acid, cannabidivarinic acid, tetrahydrocannabivarinic acid, and cannabichromevarinic acid. New spirocompounds were subsequently isolated not only from Japanese⁹³ but also Thai⁹⁴ hemp, including cannabispirenone, which gives rise to cannabispirene, cannabispirene and acetyl cannabispirene, and cannabispirenone, which could be converted to cannabispirene and cannabispirenanol. Cannabispirenenone is also source of cannabidihydrophenanthrene. A new cannabinoid, cannabinerolic acid, was isolated from a Mexican strain of *Cannabis sativa L.*⁹⁵ This cannabinoid acid is involved in the biosynthesis of Δ^1 -THCA.⁹⁶ The same authors⁹⁷ provided direct evidence for the biosynthesis of Δ^1 -THCA from CBGA through oxidocyclization by the novel enzyme Δ^1 -THCA synthase. THCA synthase is biosynthesized in the storage cavity of the glandular trichomes⁹⁸ and may be involved in the self-defense of the plant. THCA synthase has been cloned from the cDNA⁹⁹ and subsequently crystallized.¹⁰⁰

The absence of CBD and CBDA has been reported in Cannabis from South Africa^{101,102} and three regions of Mexico.¹⁰³ Why regional varieties of *Cannabis sativa L.* would produce different cannabinoids was answered in part with the proof that Δ^9 -THCA does not originate from CBDA. In 1996, the enzyme catalyzing the oxidocyclization of CBGA, as well conversion of cannabinerolic acid to CBDA, CBDA synthase, was identified.¹⁰⁴ One year later, cannabichromenic acid synthase, which catalyzes the oxidocyclization of CBGA (and cannabinerolic acid) to CBCA was identified in young leaves of *Cannabis sativa L.*¹⁰⁵ This enzyme was later purified and characterized.¹⁰⁶ Thus, the answer to the regional variability in CBD and CBDA levels is to be found in the origination of THCA and CBCA by two independent pathways from CBGA, not CBDA,⁹⁷ and the synthesis of CBDA by a third pathway.

The current status of the biogenic pathways for synthesis of cannabinoid acids in the *Cannabis sativa L.* is summarized in Figure 3.

Crombie et al.⁹⁴ isolated spiranes from Thai hemp and proposed the biogenesis of their origin. In their interpretation cannabispirenenone gives rise to cannabispirenone, further turning into cannabispirene and cannabispirenanol. Cannabispirenenone is also source of cannabidihydrophenanthrene.

Turner and El-Sohly¹⁰⁷ likewise isolated a series of polyoxidized cannabinoids as minor substances from Cannabis presenting their schema of Δ^9 -THC conversion to cannabinol in plant material through epoxy and hydroxylated cannabinoids.

Takeya and Itokawa¹⁰⁸ and Itokawa et al.¹⁰⁹ reports on biotransformation of primary and secondary allylic alcohols into the corresponding aldehydes in suspension cultures making use of the callus induced from *Cannabis sativa L.* Heitrich and Binder¹¹⁰ isolate Δ^8 -THC from the callus culture from *Cannabis sativa L.* as an artifact of the really originated either Δ^9 -THCA or Δ^9 -THC acid B.

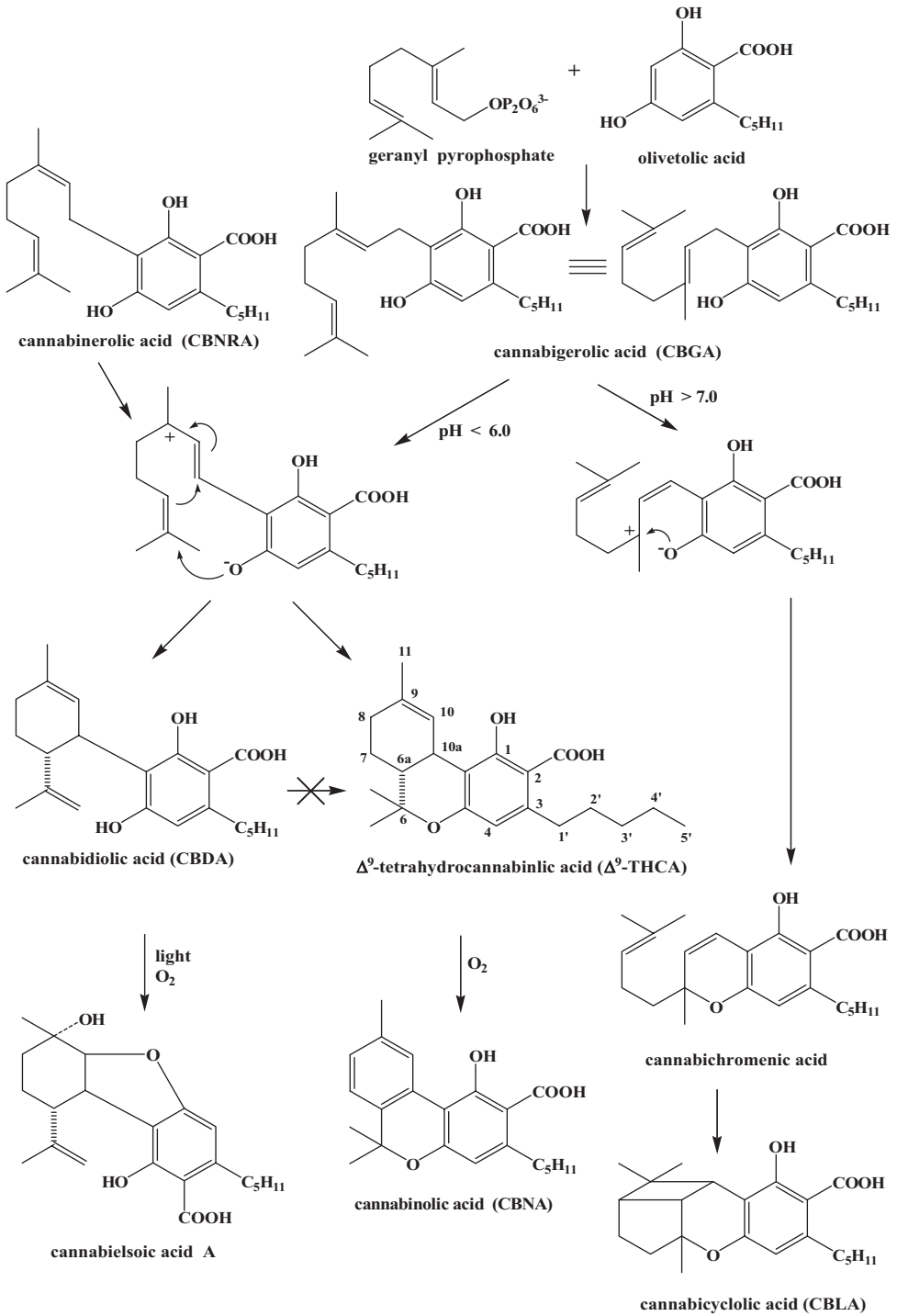


Figure 3. Proposed biogenesis of cannabinoid acids in the plant *Cannabis sativa L.*

Hartsel et al.¹¹¹ and Loh et al.¹¹² find the hemp suspensions inoculated with CBD to be producing the two principal cannabinoids, CBE C-1 diastereoisomers. The suspension cultures inoculated with olivetol produced an unidentified cannabinoid with an *m/z* 210 molecular ion.

Turner et al.¹¹³ included a newly isolated cannabiglendol in their biogenetic series starting with cannabidivanol (cannabidiol) or their acids and proceeding through cannabiglendole and cannabicitrane to Δ^8 -THC or Δ^8 -THCA. The schema is conceived generally to comprise acids and neutral cannabinoids both for the C₃ and C₅ homologues.

Kajima and Piraux¹¹⁴ utilized ¹⁴C-labeled CBG, olivetol and the olivetolic acid to study the production of cannabinoid substances in the plant. The roots of the plants were dipped in a solution containing these substances. As a result the *n*-propylcannabinoids were found not to arise through shortening of the *n*-pentyl side chain of more common cannabinoids. Decarboxylation of cannabinoid acids represents the continuous process of neutral cannabinoids production that can appear in the early stage of the plant growth and continue in the course of its vegetation. CBD and THC can arise in separate though reversible ways. During this process, the amphotern enzyme could materialize the inner transformation of CBD and vice versa. In the authors' opinion, neutral cannabinoids originate in two different processes: (a) decarboxylation of cannabinoid acids, a process probably important during the storage, (b) that one starting with neutral precursors which could appear during plant growth.

In their next study, Shoyama¹¹⁵ dealt with the biosynthesis of propylcannabinoid acids by *in vitro* incubation with raw enzyme solution from three species of *Cannabis sativa L.* The authors used cannabigevarolic acid-carboxyl-¹⁴C (giving rise to tetrahydrocannabivarolic acid and cannabichromevarinic acid), cannabidivarolic acid-carboxyl-¹⁴C (giving tetrahydrocannabivarolic acid), and cannabigerorcnic acid-carboxyl-¹⁴C (producing two products, one of them identified as the cannabichromeorcnic acid). A biogenetic schema is presented stating the relationship between methyl, propyl and pentyl cannabinoid acids.

The biogenesis of cannabinoid substances has also been the subject of Hanuš's investigation.¹¹⁶ Apart from the above mentioned article¹¹³ quotes a theoretical study conceived in a broader and more general basis. The considerations based on the analysis of known works dealing with the biosynthesis of cannabinoid substances in the plant, on the study of the cannabinoid substances isolated from various hemp species cultivated in different climatic conditions, and problems of their stability and transformation have resulted in two broadly and very generally conceived biogenetic schemas.

4. CANNABINOID RECEPTORS

A. Central (CB₁) and Peripheral (CB₂) Cannabinoid Receptors

The demonstration that Δ^9 -THC decreased prostanoid-stimulated cyclic AMP accumulation in membranes prepared from neuronally derived cells was the first indication that the effects of Δ^9 -THC may be mediated through a specific receptor.¹¹⁷ This inhibition of adenylate cyclase was stereospecific (as demonstrated with HU-210 and -211 enantiomers¹¹⁸), specific for psychoactive cannabinoids, including Δ^8 -THC¹¹⁹ and nantradol analogs,¹²⁰ and required a functional G_i protein. A high affinity, stereoselective and pharmacologically distinct cannabinoid receptor was finally identified in the rat brain in 1988.¹²¹ Several years later, cannabinoid receptor cDNA was isolated from rat cerebral cortex and human brain stem cDNA libraries, and expressed in immortalized cell lines^{122,123} The deduced amino acid sequence of the human receptor encoded a protein of 472 residues sharing 97.3% identity with the previously cloned rat cannabinoid receptor.¹²² This receptor was named CB₁. Homologues of this central cannabinoid receptor have been cloned from additional human^{123,124} and mouse¹²⁵ tissues.

A second major cannabinoid was subsequently cloned from HL-60 cells.¹²⁶ This "peripheral" cannabinoid receptor, mCB₂, was identified in macrophages residing in the marginal zone of the spleen, and was ultimately cloned from a mouse splenocyte cDNA library.¹²⁷ This protein of 347 residues shares a sequence homology of 82% with the human CB₂ (hCB₂), but is shorter than

hCB₂ by 13 amino acids at the C-terminus. The gene for the rat CB₂ receptor was cloned, expressed, and its properties compared with those of mouse and human CB₂ receptors.¹²⁸ Sequence analysis of the rat CB₂ indicates that it has a 93% amino acid identity between rat and mouse and 81% amino acid identity between rat and human. Although it was originally believed that the CB₂ receptor resided exclusively in non-CNS tissues, Van Sickle et al.¹²⁹ reported the expression of CB₂ receptor mRNA and protein localization on brainstem neurons. It is now recognized that while the CB₁ receptor is found primarily in brain and neuronal tissue, and the CB₂ receptor is expressed primarily in immunocytes,¹³⁰ both the CB₂ receptor and its gene transcripts are widely distributed throughout the mammalian brain.^{131,132} Both the CB₁ and CB₂ receptors are coupled through G proteins to adenylyl cyclase and mitogen-activated protein kinases. CB₁ receptors are also coupled through G proteins to several types of calcium and potassium channels.

B. GPR55 Receptor

A unique receptor that mediates the mesenteric vasodilator response to anandamide and R-methanandamide has been reported.¹³³ This receptor is located on endothelial cells, and is distinct from the CB₁ in that it is not activated by other cannabinoids, but can be inhibited by the CB₁-selective antagonist SR141716A. This receptor is of potential therapeutic interest as activation of endothelial anandamide receptors may contribute to mesenteric vasodilation in endotoxic shock.

Insight into the identity of this receptor was provided in 1999 by Sawzdargo et al.,¹³⁴ who reported the identification and cloning of two novel human intronless G protein-coupled receptor genes, GPR52, GPR55, and a pseudogene ΨGPR53. GPR55 was identified from the expressed sequence tags database. A partial cDNA clone obtained from the IMAGE Consortium of GPR55 was used to screen a human genomic library to acquire the full length gene. GPR55 encode receptors of 319 amino acids, and the GPR55 gene was mapped to chromosome 2q37 using fluorescence *in situ* hybridization (FISH). Its mRNA transcripts have been detected in the caudate nucleus and putamen, but not in five other brain regions. Human receptors showing the highest amino acid identity to GPR55 include P2Y5 (29%), GPR23 (30%), GPR35 (27%), and CCR4 (23%).

GPR55 is a novel receptor that binds and is activated by a number of endogenous, natural and synthetic cannabinoid ligands,^{135,136} including: anandamine (EC₅₀ = 18.4 ± 4.1 nM); noladin ether (11.5 ± 0.5 nM); palmitoylethanolamide (3.2 ± 1.3 nM); virodhamine (10.4 ± 1.4 nM); 2-arachidonylglycerol (2-AG) (3.5 ± 2.4 nM); Δ⁹-THC (8.9 ± 1.1 nM); JWH-015 (4.75 ± 0.25 nM); and CP55940 (5 ± 1 nM).¹³⁷ This receptor is expressed in several tissues and may play a role not only in vascular function, but lipid metabolism, as GPR55 appears to be a functional receptor for lysophosphatidylinositol.¹³⁸ Another novel ligand for this receptor may be *N*-arachidonoyl-L-serine (ARA-S), which was recently isolated from bovine brain.¹³⁹ This compound has low affinity for the CB₁, CB₂ or vanilloid TRPV1 receptors. However, it produces endothelium-dependent vasodilation of isolated rat mesenteric arteries and abdominal aorta, paralleling the effects of abnormal-CBD (Abn-CBD), a synthetic agonist of a putative cannabinoid-type receptor.^{140,141} Additional studies will be necessary to determine if ARA-S is another endogenous agonist for this receptor. Moreover, additional investigation according to the guidelines of Pertwee¹⁴² will be necessary to determine if GPR55 and its apparent endogenous ligands represent a new endogenous cannabinoid system.

5. ENDOCANNABINOIDS

The discovery of the CB₁ and CB₂ G-protein coupled cannabinoid receptors suggested the existence of endogenous ligand(s), which could bind to these receptors and exert a physiological effect. A list of the currently known endocannabinoids is presented in Figure 4. Interest in describing these endocannabinoid(s) led Mechoulam's group^{143,144} to isolate and identify the first endogenous cannabinoid from porcine brain, which was named anandamide. This endocannabinoid inhibited the specific binding of a radiolabeled cannabinoid probe to synaptosomal membranes in a manner typical

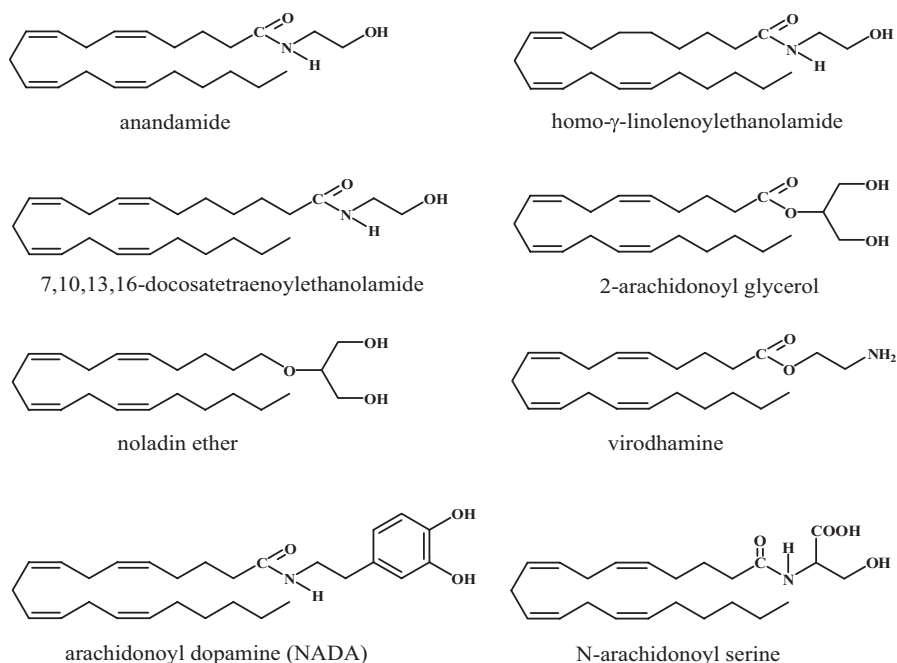


Figure 4. Known endocannabinoids.

of competitive ligands and produced a concentration-dependent inhibition of the electrically evoked twitch responses of the mouse vas deferens, a characteristic effect of psychotropic cannabinoids. For the first known mass spectrum of anandamide and its TMS-derivative (original spectra of that time) see Figure 5.

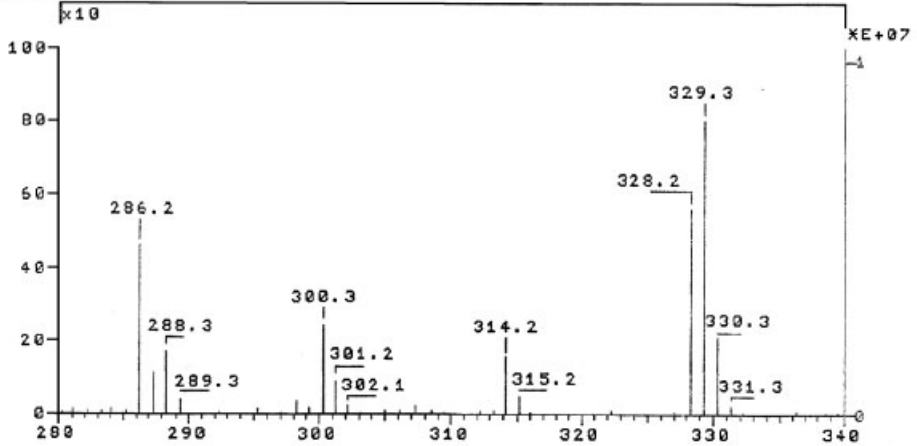
Our research group expected that additional polyunsaturated fatty acid ethanolamides may be present, and subsequently identified in porcine brain two putative endocannabinoids, homo- γ -linolenylethanolamide (CB_1 ; $K_i = 53.4 \pm 5.5$ nM) and 7, 10, 13, 16-docosatetraenylethanolamide (CB_1 ; $K_i = 34.4 \pm 3.2$ nM).¹⁴⁵ A compound with the same molecular weight as anandamide was identified as O-arachidonoyl ethanolamine (arachidonic acid and ethanolamine joined by an ester linkage) ($EC_{50} = 1,906$ nM). Based on the opposite orientation of its components relative to arachidonylethanolamide, the molecule was named virodhamine from the Sanskrit word *virodha*, which means opposition.¹⁴⁶

Subsequently, a series of structurally novel classes of endocannabinoids were isolated. The first was an ester, 2-AG (CB_1 ; $K_i = 5.85 \pm 0.12$ μ M).^{147,148} The third major class of endocannabinoid is the ether-type, represented by 2-arachidonoyl glyceryl ether (noladin ether). The structure of noladin ether was determined by mass spectrometry and nuclear magnetic resonance spectroscopy and was confirmed by comparison with a synthetic sample. It binds with relatively high affinity to the CB_1 receptor ($K_i = 21.2 \pm 0.5$ nM), but weakly to the CB_2 receptor ($K_i > 3$ μ M). Administration of 2-arachidonoyl glyceryl ether causes sedation, hypothermia, intestinal immobility, and mild antinociception in mice.¹⁴⁹

Because of its structural similarity to anandamide, ARA-S¹³⁹ was believed to represent another endogenous ligand for the CB receptors. However, ARA-S was found to bind very weakly to the CB_1 , CB_2 or vanilloid TRPV1 receptors. Because it produces endothelium-dependent vasodilation of rat isolated mesenteric arteries and abdominal aorta, and stimulates phosphorylation of p44/42 MAP kinase and protein kinase B/Akt in cultured endothelial cells, it was believed to be the natural ligand of the GPR55 (see Section 5.2). ARA-S also suppresses LPS-induced formation of tumor necrosis factor- α (TNF- α) in a murine macrophage cell line and in wild-type mice, as well as in mice deficient

A

SPEC: HU530FSL ver 8 on UIC 4 4 13-MAY-92 DERIVED SPECTRUM 9
 Samp: Start : 14:23:26 10
 Coan: HU530
 Mode: EI +Q1MS LMR UP LR
 Oper:
 Base: 85.1 Inten : 10373607 S Inlet : GC
 Norm: 85.1 RIC : 70388752 Masses: 50 > 700
 Peak: 1000.00 mmu # peaks: 462
 Data: ! 349



B

SPEC: HU530FS1 ver 2 on UIC 004004 14-MAY-92 DERIVED SPECTRUM 9
 SAMP: Start : 00:53:43 10
 COAN: LUMIR SAMPLE 530 SILYLATED
 Mode: EI +Q1MS LMR UP LR
 Oper:
 Base: 73.0 Inten : 3829520 Inlet : GC
 Norm: 73.0 RIC : 73122224 Masses: 50 > 700
 Peak: 1000.00 MMU # peaks: 506
 Data: ! 524

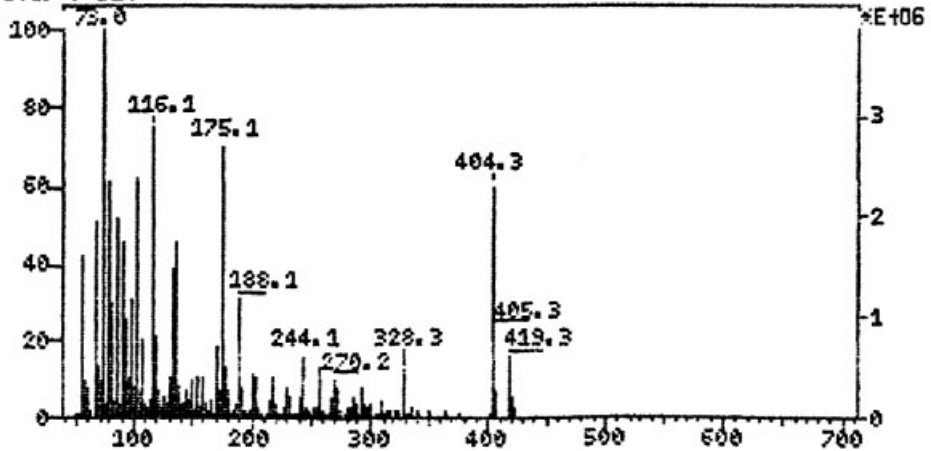


Figure 5. A: First known original mass spectrum of anandamide (HU-530) recorded by dr. Asher Gopher (May 13, 1992). **B:** First known original mass spectra of anandamide (HU-530) after silylation recorded by Dr. Asher Gopher (May 14, 1992).

in CB₁ or CB₂ receptors. Many of these effects parallel those reported for Abn-CBD, a synthetic agonist of a putative novel cannabinoid-type receptor.^{140,141}

While the above endocannabinoids appear to have affinity for the primary CB₁ and CB₂ receptors, little is known of endogenous ligands for the related vanilloid receptors. Huang et al.¹⁵⁰ hypothesized that N-arachidonoyl-dopamine (NADA) may exist as an endogenous “capsaicin-like” ligand for the VR1 in mammalian nervous tissues. They found that NADA is an endocannabinoid, and can be found in the CNS, with high concentrations in the striatum, hippocampus, and cerebellum and lower concentrations in the dorsal root ganglion. NADA binds preferentially to the CB₁ receptor, with a 40-fold selectivity ($K_i = 250 \pm 130$ nM) over the CB₂ receptors. While NADA activates TRPV1 receptors, it is a partial agonist in tissues with low receptor reserves, but a full agonist in tissues with high receptor reserve and in certain disease states.¹⁵¹ NADA potently ($EC_{50} = 40 \pm 6$ nM) activates the TRPV1, which is only activated by both capsaicin and endogenous arachidonic acid derivatives, including anandamide.¹⁵² It has also been demonstrated that NADA and related compounds are good inhibitors of fatty acid amide hydrolase (FAAH). Given that NADA has strong cannabimimetic effects both *in vitro* and *in vivo*, but has low affinity for the CB receptors, the cannabimimetic actions of NADA could result from its inhibition of the inactivation of anandamide.¹⁵³

6. BIOSYNTHESIS AND DEGRADATION OF ENDOCANNABINOIDS

The biosynthesis and metabolism of the endocannabinoids have been discussed in detail in numerous reviews (Figs. 6 and 7).^{154–159}

Anandamide is formed following a pathway previously proposed for other fatty acid ethanolamides, namely the initial formation of *N*-acylphosphatidylethanolamine (NAPE). At least three independent pathways for anandamide formation from NAPE have been observed,¹⁶⁰ including

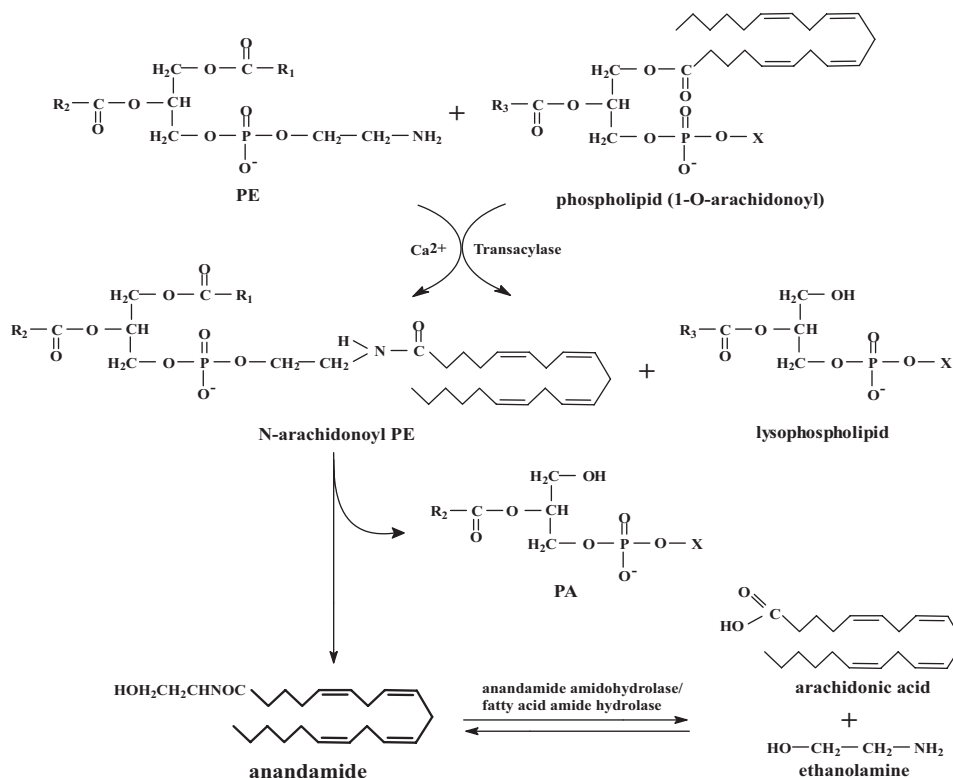


Figure 6. Pathways for the biosynthesis and degradation of anandamide.

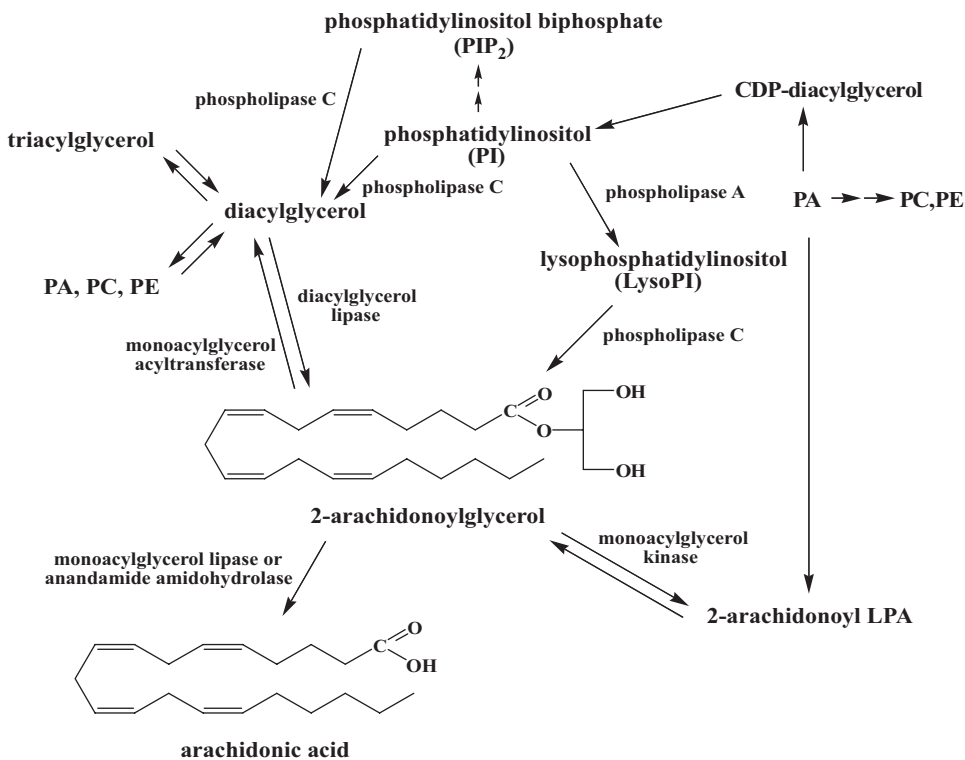
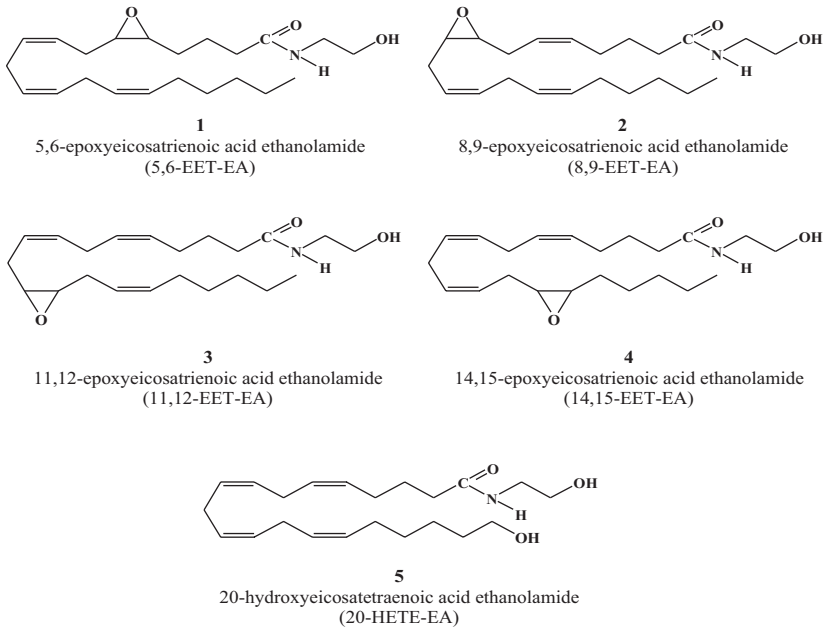


Figure 7. Pathways for the biosynthesis and degradation of 2-arachidonoylglycerol (2-AG).

the transacylation-phosphodiesterase pathway (Figs. 8 and 9).^{161–164} In animal tissues, NAPE is catalyzed by a Ca^{2+} -stimulated and membrane-associated *N*-acyl-transferase that transfers a fatty acyl chain from the *sn*-1 position of glycerophospholipids to the primary amine of phosphatidylethanolamide.^{165,166} *N*-acylphosphatidylethanolamides are precursors of bioactive *N*-acylethanolamides, including anandamide.¹⁵⁹ Cadas et al.¹⁶⁷ described a *N*-acyltransferase activity in the brain catalyzing the biosynthesis of *N*-arachidonoyl PE (phosphatidylethanolamide) by transferring an arachidonate group from the *sn*-1 carbon of phospholipids to the amino group of PE. They also show that *sn*-1 arachidonoyl phospholipids are present in brain. In addition to their function as endocannabinoid precursors, NAPEs and *N*-acylethanolamides (NAEs) may act as neuroprotectives, accumulating in the brain in response to trauma/hypoxia, during which time other phospholipids are rapidly degraded. Their neuroprotective activity may be mediated through both CB receptor and nonreceptor-mediated effects, such as interfering with ceramide turnover.¹⁶⁸

Based on the functional similarity of NAT to lecithin-retinol acyltransferase (LRAT), Jin et al.,¹⁶⁹ examined phosphatidylethanolamide *N*-acylation activity in two rat LRAT homologous proteins. Their results reveal that rat LRAT-like protein (RLP-1) functions as a phosphatidylethanolamide *N*-acyltransferase, catalytically distinguishable from the known Ca^{2+} -dependent NAT. Results of Astarita et al.¹⁷⁰ reveal a previously unrecognized preference of brain *N*-acyl transferase activity for polyunsaturated *N*-arachidonoyl phosphatidylethanolamide (NarPE) and provide new insights on the physiological regulation of anandamide biosynthesis. They characterized the precise molecular composition of NarPE species in the rat brain and demonstrated that Ca^{2+} potently enhances the formation of a subset of such species, presumably through the stimulation of a selective *N*-acyl transferase activity.

The metabolism of anandamide by human liver and kidney microsomes and the formation of epoxyeicosatrienoic ethanolamides and hydroxyeicosatetraenoic acid ethanolamide was



studied and described.¹⁷¹ P450 3A4 plays a role in the formation of 5,6-, 8,9-, 11,12-, and 14, 15-epoxyeicosatrienoic acid ethanolamides (**1–4**). A potential role for P450 4F2 in the formation of 20-hydroxyeicosatetraenoic acid ethanolamide (**5**) in both liver and kidney is demonstrated. The epoxyeicosatrienoic acid ethanolamides produced by the liver microsomal P450s were converted to their corresponding dihydroxy derivatives by microsomal epoxide hydrolase.

Affinity of oxygenated metabolites of anandamide and 2-AG for CB₁ and CB₂ receptors was studied.¹⁷² Oxidative metabolism of endocannabinoids, anandamide and 2-AG (see Fig. 10), by

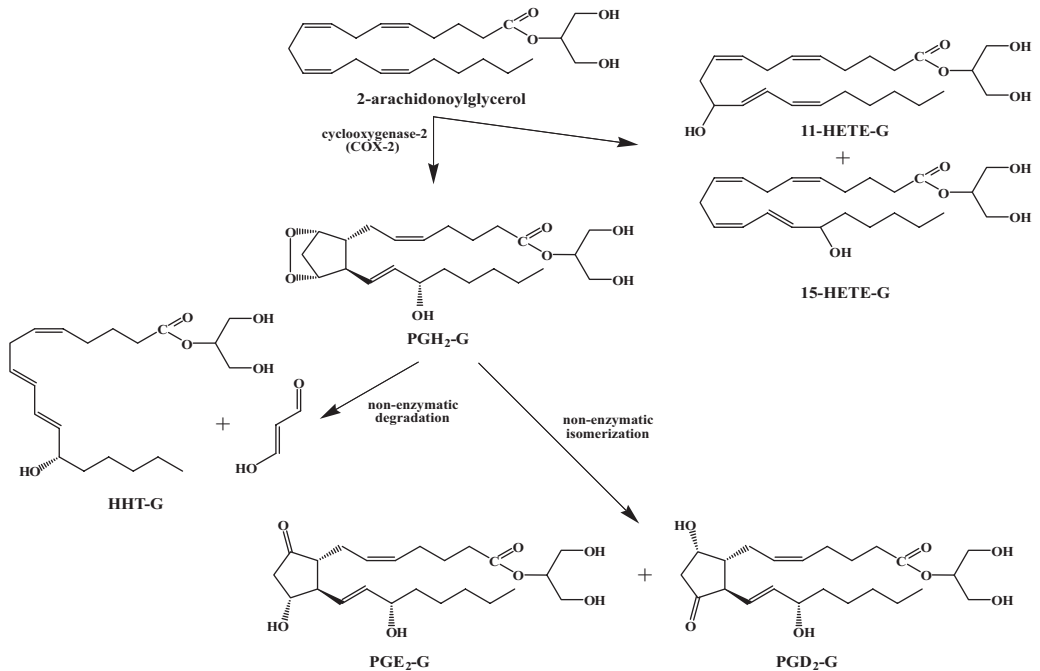


Figure 10. Oxidative metabolism of anandamide and 2-arachidonoylglycerol by cyclooxygenase-2.

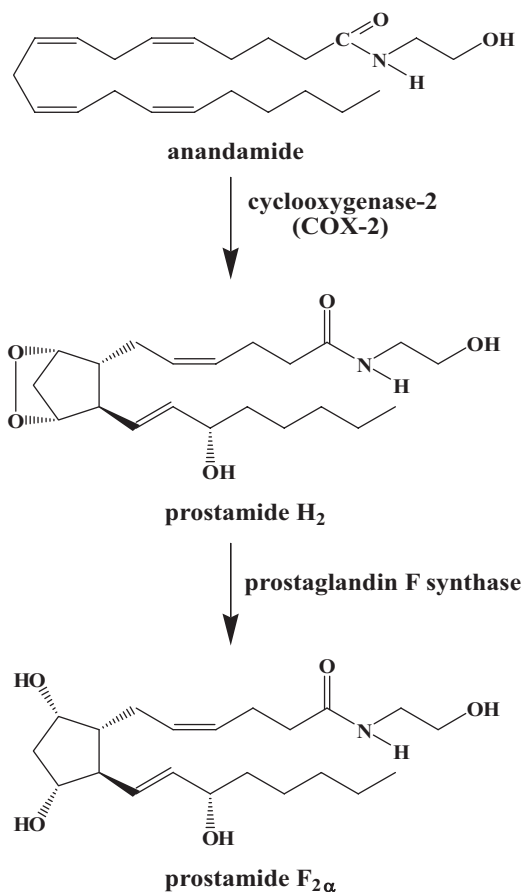


Figure 11. The biosynthesis of prostamide F_{2α} from anandamide.

cyclooxygenase-2 was studied intensively recently.^{173–176} The biosynthesis of prostamide F_{2α} from anandamide was published.¹⁷⁷ Prostamide H₂ as a possible intermediate of anandamide metabolism was identified during a series of metabolic studies with the help of recombinant human COX-2 and prostaglandin F synthase (Fig. 11).

Primary cultures of neurons contain detectable levels of NAPE, which is synthesized in response to elevations in intracellular calcium levels and the actions of a protein kinase. Enzymatic hydrolysis of NAPE by phospholipase D yields anandamide. Anandamide is not stored in cells but is formed mainly when needed, then released from neurons on depolarization and rapidly inactivated. Similarly, 2-AG is synthesized in response to an influx of calcium into cells. Enzymatic hydrolysis of diacylglycerol (DAG) seems to be the most important route,¹⁵⁹ although phospholipase C catalyzed hydrolysis of phosphatidylcholine or phosphatidylinositol to yield 2-AG has also been reported. The reuptake of 2-AG is partly inhibited by other endogenous acylglycerols and is part of the “entourage” effect (see below).

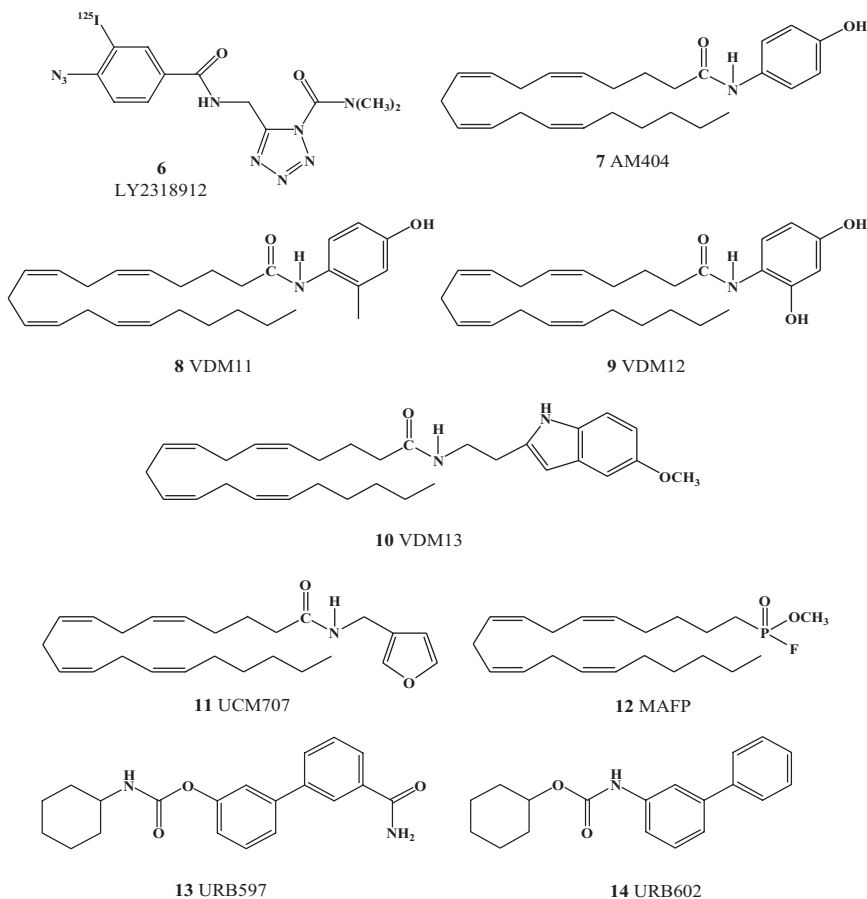
While the mechanisms of endocannabinoid inactivation are not completely understood, it appears to involve a two step process, where it is actively imported into cells where it is degraded by FAAH. A potent, competitive small molecule inhibitor of anandamide uptake (LY2318912, IC₅₀ 7.27 ± 0.51 nM; **6**) was used to identify a high-affinity, saturable anandamide transporter binding site (K_d = 7.62 ± 1.18 nM) that is distinct from fatty acid amide hydrolase.^{178,179} In attempts to increase anandamide levels indirectly, a number of agents have been developed which suppress anandamide degradation. Anandamide transporter inhibitors include *N*-(4-hydroxy-

phenyl)-arachidonylethanolamide (AM404, **7**)^{180,181} and several derivatives of cannabidiol.¹⁸² AM404 inhibits high-affinity anandamide accumulation in rat neurons and astrocytes *in vitro*, suggesting that this accumulation results from carrier-mediated transport. Novel anandamide derivatives, VDM11 (**8**), VDM12 (**9**), and VDM13 (**10**), have been found which inhibit the anandamide membrane transporter (AMT) as potently as AM404, inhibit monoacylglycerol lipase (MAGL), while exhibiting little or no agonist activity at the hVSR1.^{183,184} These compounds only weakly inhibit anandamide hydrolysis and are poor CB₁/CB₂ receptor ligands. A more selective and potent inhibitor of anandamide uptake *in vivo* is UCM707 (**11**),¹⁸⁵ which shows a negligible interaction with cannabinoid receptors, while potentiating endocannabinoid activity.¹⁸⁶ UCM707 potentiated the hypokinetic and antinociceptive effects of a subeffective dose of anandamide more specifically than previously described AMT inhibitors.

For a recent review on the cellular transport of endocannabinoids and its inhibition, see Fowler and Jacobsson.¹⁸⁷

Once within the cell, anandamide and 2-AG are hydrolyzed to arachidonic acid and ethanolamine, or glycerol, respectively, by the fatty acid amide hydrolase (FAAH).¹⁸⁸ FAAH has fairly broad specificity, as it also hydrolyses oleamide, a sleep inducing factor.^{189,190}

The first potent and relatively selective inhibitor of anandamide catabolism is methylarachidonylfluorophosphonate (MAFP, **12**).¹⁹¹ MAFP and related analogs inhibit FAAH catalyzed hydrolysis of anandamide,¹⁹² but also blocks arachidonylethanolamide amidase. Indeed, MAFP was found to irreversibly inhibit the CB₁ receptor, complicating interpretations of its activity.¹⁹³ In addition to FAAH, 2-AG is catabolised by monoacylglycerol lipase (MAGL). The presence of



MAGL activity in microglial cells¹⁹⁴ suggest the ability of these cells to regulate 2-AG levels in the CNS. However, the ability of the mouse microglial cell line BV-2, which lacks MAGL, to hydrolyze 2-AG suggests the involvement of other lipases. URB597 (3'-carbamoyl-biphenyl-3-yl-cyclohexylcarbamate, **13**) reduces 2-AG this hydrolysis 50%, consistent with the involvement of FAAH. The remaining activity is blocked by MAGL inhibitors [[1,1-biphenyl]-3-yl-carbamic acid, cyclohexyl ester (URB602, **14**) and MAFP (methylarachidonyl fluorophosphate)], and is unaffected by inhibitors of cyclooxygenases (COXs), lipooxygenases (LOXs), and diacylglycerol lipases (DGLs), indicating the involvement of a novel MGL activity.

7. THE ENDOCANNABINOID CONGENERS: MEDIATORS OF THE ENTOURAGE EFFECT

Production of endocannabinoids in biological systems yields saturated and mono- or diunsaturated congeners (monoacylglycerols and N-acylethanolamides) that are inactive at CB receptors, but which nonetheless influences endocannabinoid metabolism, for example, by inhibiting AMT or FAAH, or interact with non-CB receptors (Fig. 12). Palmitoylethanolamide exhibits anti-inflammatory and analgesic activity, even though it does not activate CB₁ or CB₂ receptors.^{195–197} Palmitoylethanolamide binds to and potently activates (EC₅₀ = 3.2 ± 1.3 nM) GPR55 (which may be a new cannabinoid receptor), which may mediate its pharmacologic activity.¹³⁵ Production of 2-AG in the spleen, brain and gut is accompanied by several 2-acyl glycerol esters, including 2-linoleoyl glycerol and 2-palmitoyl glycerol. These compounds significantly potentiate the binding of 2-AG to CB receptors, and its ability to inhibit adenylyl cyclase. The biological activity of 2-AG can also be increased by related, endogenous 2-acyl glycerols, which show no significant activity by themselves. This “entourage effect” may represent a novel route for molecular regulation of endogenous cannabinoid activity.¹⁹⁸

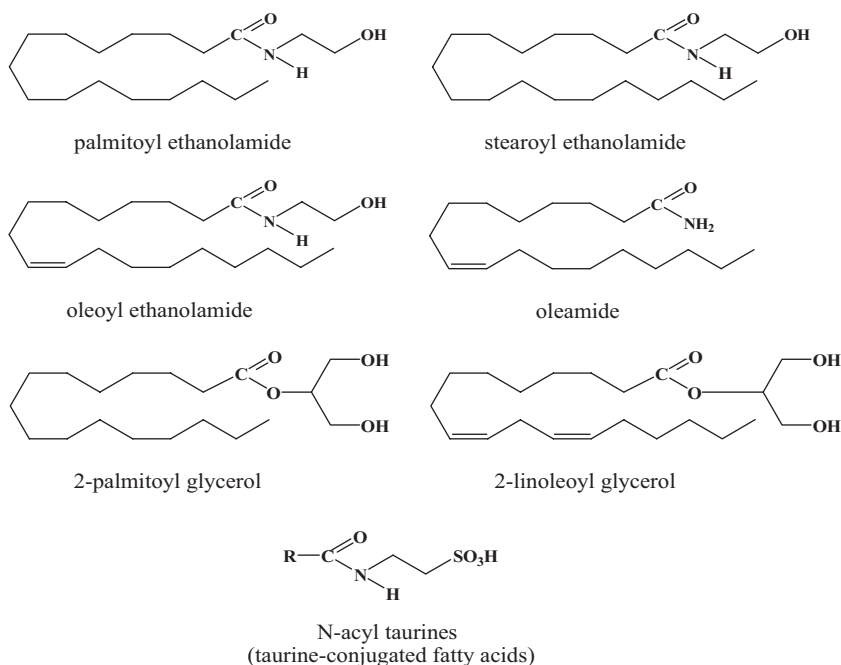


Figure 12. Known endocannabinoids congeners.

Another saturated ethanolamide, stearoylethanolamide, exerts a marked dose-dependent anorexic effect. This congener reduces food intake in mice in a structurally selective manner,¹⁹⁹ and binds to a unique site unrelated to known cannabinoid or vanilloid receptors.²⁰⁰ Moreover, this site is not coupled to G proteins, regulating different signaling pathways. The pro-apoptotic activity of stearoylethanolamide is regulated by NO in a way opposite to that reported for anandamide. Stearoylethanolamide potentiates the decrease of cAMP induced by AEA in mouse cortical slices, suggesting that it acts through an “entourage” effect.²⁰¹

Oleoylethanolamide is an endogenous regulator of food intake, and intraperitoneal injection of this compound decreased food intake in 24 hr-starved rats.²⁰² This endogenous lipid mediator reduces food intake (the satiating factor) and decreases body weight gain in rodents by activating the nuclear receptor peroxisome proliferator-activated receptor- α . Oleoylethanolamide has a central and peripheral anorexic effect. It is a naturally occurring bioactive lipid with hypophagic and anti-obesity effects.²⁰³ A possible protective action of oleoylethanolamide against reactive oxygen species, could explain its beneficial effects on *in vitro* capacitated spermatozoa.²⁰⁴

Oleamide, an unsaturated fatty acid amide which can modulate central nervous system function was isolated from the cerebrospinal fluid of sleep deprived cats²⁰⁵ and rats.^{206,207} However, the mechanism by which oleamide induces sleep is poorly understood. It does not bind with high affinity to CB₁ or CB₂ receptors, but exhibits cannabimimetic actions which could be explained at least in part by entourage effects, such as FAAH inhibition. Oleamide is a full cannabinoid CB₁ receptor agonist.²⁰⁸ Oleamide relaxed small mesenteric arteries in the rat, an effect dependent on the presence of the endothelium, activation of Ca²⁺-sensitive K⁺ channels and capsaicin-sensitive sensory nerves.²⁰⁹ Nonetheless, oleamide reportedly interacts with other receptor systems, including 5-HT_{2A} receptors and GABA receptors, but the possible existence of specific receptors for this compound is open.²¹⁰

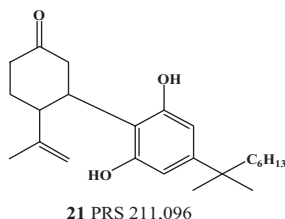
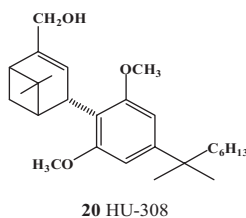
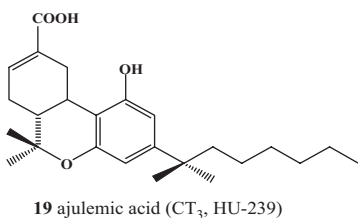
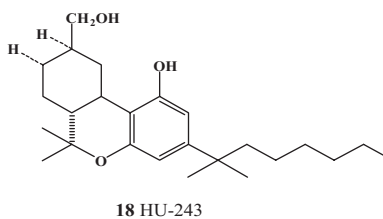
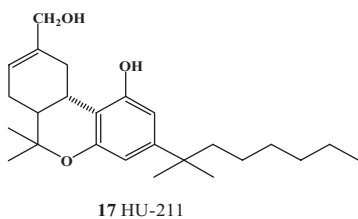
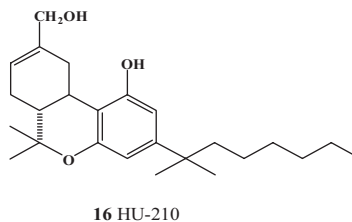
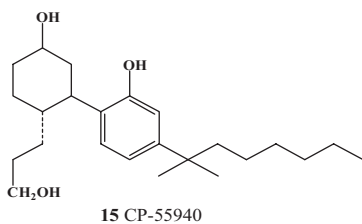
Several brain lipids are regulated by the mammalian enzyme fatty acid amide hydrolase *in vivo*, including a novel family of nervous system-enriched natural products, the taurine-conjugated fatty acids were discovered recently.²¹¹ N-acyl taurines were found to activate multiple members of the transient receptor potential family of calcium channels, including TRPV1 and TRPV4, which are both expressed in kidney. The dramatic elevation in endogenous levels of N-acyl taurines following acute or chronic inactivation of FAAH, in conjunction with the pharmacological effects of these lipids on TRP channels, suggests the existence of a second major lipid signaling system regulated by FAAH *in vivo*.²¹²

8. SYNTHETIC CANNABINOID AND NONCANNABINOID AGONISTS/ANTAGONISTS OF CANNABINOID RECEPTORS

A. Synthetic Cannabinoid Analogues

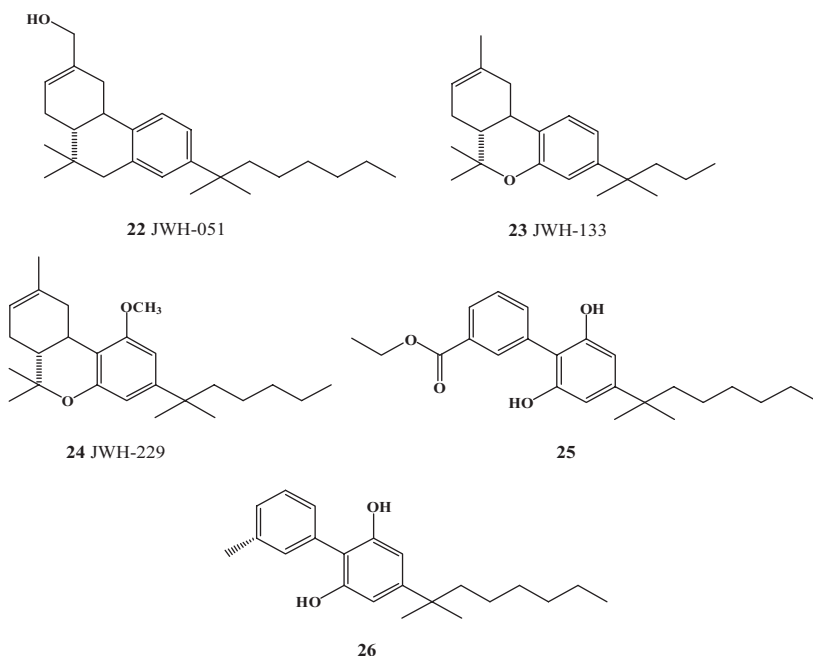
In an attempt to produce novel analgesics, Pfizer produced a number of bicyclic compounds active at the CB receptor. The compound chosen for clinical evaluation was CP-55,940 (**15**), which was significantly more potent than morphine.^{213–215} Although highly active analogs were produced, the cannabinoid-type side effects observed with this series of nonreceptor selective, “non-classical” cannabinoids led to the termination of the project.²¹⁶ Nonetheless, these compounds were widely used as labeled ligands,²¹⁷ ultimately aiding in the identification of the first cannabinoid receptor.¹²¹

Subsequently, a number of agents were created with high affinity and stereospecificity.²¹⁸ Replacing the n-pentyl with a 1,1-dimethyl heptyl side chain of the primary active metabolite of Δ^8 -THC, 11-hydroxy- Δ^8 -THC, led to HU-210 (**16**) and its psychotropically inactive enantiomer, HU-211 (**17**). Interestingly, both compounds are analgetic and antiemetic, and HU-211 is presently being evaluated as an anti-trauma agent. Both compounds were synthesized with very high enantiomeric purity (99.8%).²¹⁹ The high degree of enantioselectivity and potency of HU-210



was demonstrated in mice, dogs and pigeons.^{220,221} HU-210 was hydrogenated to yield two epimers of 5'-(1,1-dimethylheptyl)-7-hydroxyhexahydrocannabinol.²²² The equatorial epimer (designated as HU-243, **18**) is the highest affinity cannabinoid receptor ligand, binding with a K_D of 45 pM. Additional efforts to increase therapeutic activity while reducing the psychological side effects led to the preparation of ajulemic acid (HU-239, **19**), which binds to CB (CB₁, K_i = 480.6 nM in rat brain synaptosomal membranes; CB₂, K_i = 170.5 nM in COS cells) and is as active as THC in the mouse tetrad assay.²²³ Additionally, HU-239 is active as an analgesic, antiinflammatory, and antineoplastic agent.^{224–227} However, it binds to the peroxisome proliferator-activated receptor γ (PPAR γ)²²⁸ and induces apoptosis in human T lymphocytes, suggesting that not all of its *in vivo* activity is mediated through cannabinoid receptors.²²⁹ While these agents are either unselective or CB₁ selective, the structurally related compound HU-308 (**20**) is CB₂-selective.²³⁰ Both of the phenolic groups in HU-308 are blocked with methyl ethers, in contrast to cannabinoid CB₁ agonists in which at least one of the phenolic groups is free. This agent has anti-inflammatory activity, and possesses no significant CNS side effects, presumably due to its lack of CB₁ receptor affinity. Other CB₂ receptor selective agents, including PRS-211096 (**21**), are being investigated for their immunomodulatory effects, including indications for the treatment of multiple sclerosis.²³¹

SAR have indicated that the cannabinoid side chain and the phenolic hydroxyl are key elements in CB₁ receptor recognition. To test this hypothesis, the 1-deoxy analogue, JWH-051 (**22**), of the very potent cannabinoid 11-hydroxy- Δ^8 -THC-DMH (HU-210) was prepared and the affinity of this

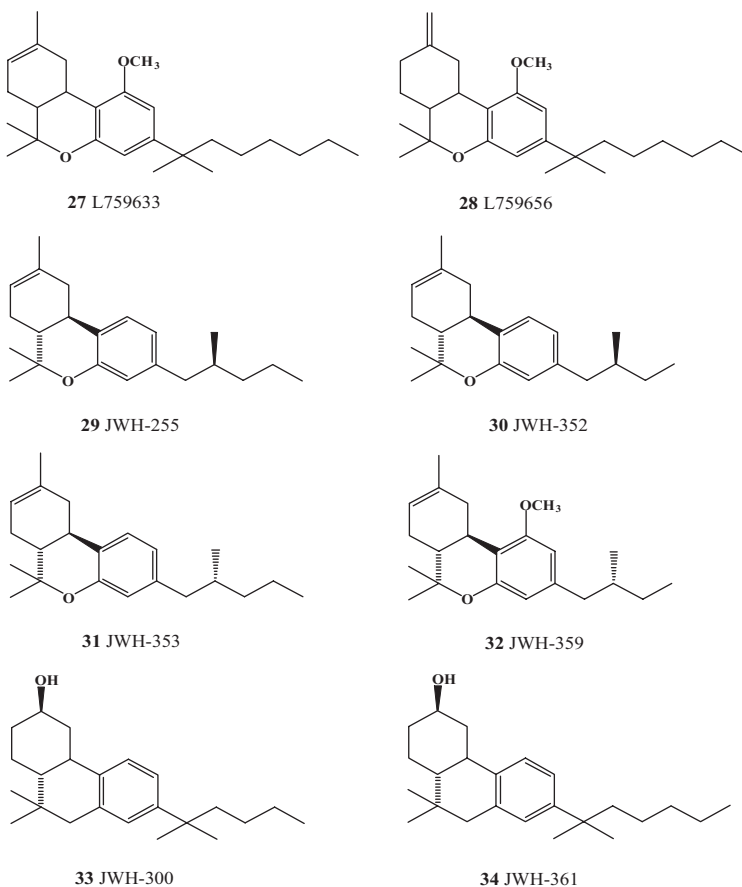


compound for the CB₁ receptor determined.²³² Contrary to expectations, this 1-deoxy analogue had high affinity for the CB₁ receptor ($K_i = 1.2 \pm 0.1$ nM) and even greater affinity for the CB₂ receptor ($K_i = 0.032 \pm 0.19$ nM). Based on this data, it is apparent that a phenolic hydroxyl group is not essential for cannabinoid activity. To obtain selective ligands for the CB₂ and to explore the structure-activity receptor of the 1-deoxy-cannabinoids, fifteen 1-deoxy- Δ^8 -THC analogues were prepared and analyzed.²³³ Five of these analogues interacted with high affinity ($K_i \leq 20$ nM) for the CB₂ receptor, and four of which had low affinity ($K_i \geq 295$ nM) for the CB₁ receptor. Of all the compounds in this series tested, 3-(1',1'-dimethylbutyl)-1-deoxy- Δ^8 -THC (JWH-133, **23**) had the highest affinity for the CB₂ receptor ($K_i = 3.4 \pm 1.0$ nM) and low affinity for the CB₁ receptor ($K_i = 677 \pm 132$ nM).

In view of the importance of the CB₂ receptor as a therapeutic target with reduced side-effect profile, three CB₂ selective cannabinoid receptor ligands, 1-methoxy-, 1-deoxy-11-hydroxy-, and 11-hydroxy-1-methoxy- Δ^8 -tetrahydrocannabinols, were synthesized.²³⁴ These compounds have greater affinity for the CB₂ receptor than for the CB₁ receptor, however only 1-methoxy-3-(1',1'-dimethylhexyl)- Δ^8 -THC (JWH-229, **24**) had essentially no affinity for the CB₁ receptor ($K_i = 3,134 \pm 110$ nM) with high affinity for CB₂ ($K_i = 18 \pm 2$ nM). A series of biphenyls were synthesized as CB₂ selective modulators.²³⁵ Compound **25** binds to CB₂ with a K_i value of 0.8 nM and to CB₁ with a K_i value of 241 nM. These nonclassical cannabinoids appear to be effective in models of peripheral pain, neuropathy, and neurodegenerative diseases.

A series of resorcinol derivatives were developed as selective CB₂ agonists.²³⁶ Compound **26** had K_i values of 40 and 0.8 nM, respectively, for the CB₁ and CB₂ receptors. In addition, this compound was assessed by intravenous administration and exhibited ED₅₀ values of 2.7, 2.4, and 3.6 mg/kg in the spontaneous activity, tail-flick and rectal temperature assays, respectively. CB₂ selective ligands, L759633 (**27**) and L759656 (**28**), that behave as potent, high-efficacy agonists, were characterized.²³⁷

New series of cannabinoid ligands for the CB₂ receptor were prepared.²³⁸ All prepared ligands have greater affinity for the CB₂ receptor than the CB₁ receptor. Four of these compounds, JWH-255 (**29**), JWH-352 (**30**), JWH-353 (**31**), and JWH-359 (**32**), have good activity for CB₂ receptor (24 nM,

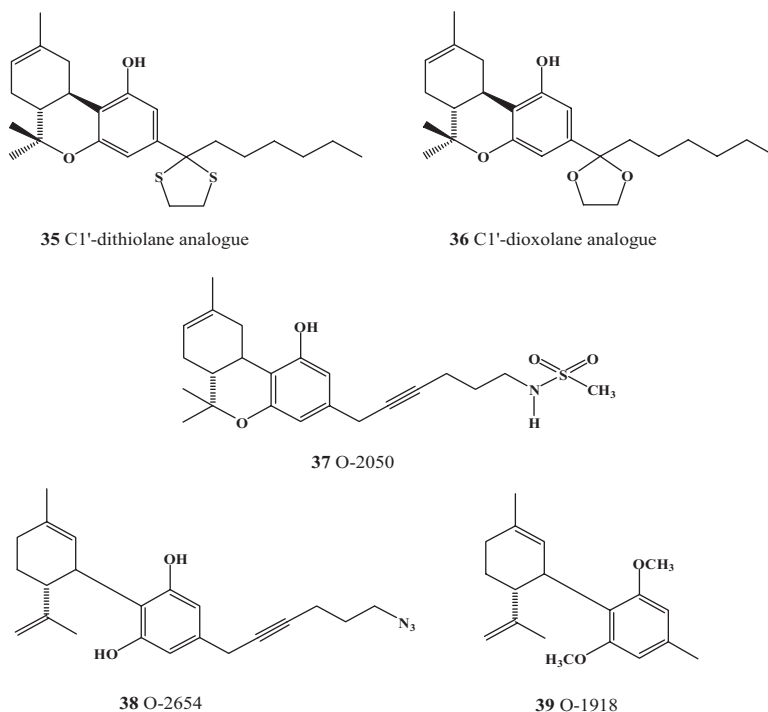


31 nM, 47 nM, and 13 nM) and weak affinity for the CB₁ receptor (4.3 μM, 1.5 μM, > 10 μM, and 2.9 μM).

11-nor-1-methoxy-9-hydroxyhexahydrocannabinols and 11-nor-1-deoxy-9-hydroxyhexahydrocannabinols are new selective ligands for the CB₂ receptors.²³⁹ From 14 prepared novel selective cannabinoids very high CB₂ affinity was determined for JWH-300 (**33**) (5.3 nM vs. 118 nM for CB₁ receptor) and JWH-361 (**34**) (2.7 nM against 63 nM for CB₁).

A successful attempt to conduct a molecular modeling and NMR-based 3D-QSAR CoMFA and CoMSIA studies of the CB₁ and CB₂ agonist pharmacophore models presented Durdagi et al.²⁴⁰ A set of 30 novel Δ⁸-THC and CBD analogues were studied. C1'-dithiolane (**35**) and C1'-dioxolane (**36**) analogues of Δ⁸-THC showed high activity but low selectivity for the CB₁ (K_i = 0.32 respectively 0.52 nM) and CB₂ (K_i = 0.52 resp. 0.22 nM) receptors attributed to their fit in the hydrophobic subsite of both receptors. Δ⁸-THC analogues had higher binding affinities than their respective CBD analogues.

Martin et al.^{241,242} prepared “silent” antagonist, O-2050 (**37**). This compound exhibited high CB₁ receptor affinity, but failed to produce either antinociception or hypothermia. Results of Gardner and Mallet²⁴³ with this compound support the notion that cannabinoid receptor antagonists suppress feeding behavior by blocking an endogenous cannabinoid orexigenic signal, rather than by inverse agonism at cannabinoid receptors. Similar compound, O-2654 (6''-azidohept-2''-yne-cannabidiol, **38**), was prepared by Thomas et al.²⁴⁴ This ligand is a competitive cannabinoid CB₁ receptor antagonist. Since it did not enhance the amplitude of electrically evoked contractions, it may be a neutral cannabinoid CB₁ receptor antagonist.



Finally neutral antagonist, O-1918 (**39**), prepared and studied Offertaler et al.¹⁴⁰ This cannabidiol analog does not bind to CB₁ or CB₂ receptors and does not cause vasorelaxation, it does cause concentration-dependent inhibition of the vasorelaxant effects of abnormal-CBD and anandamide. O-1918 is a selective, silent antagonist.

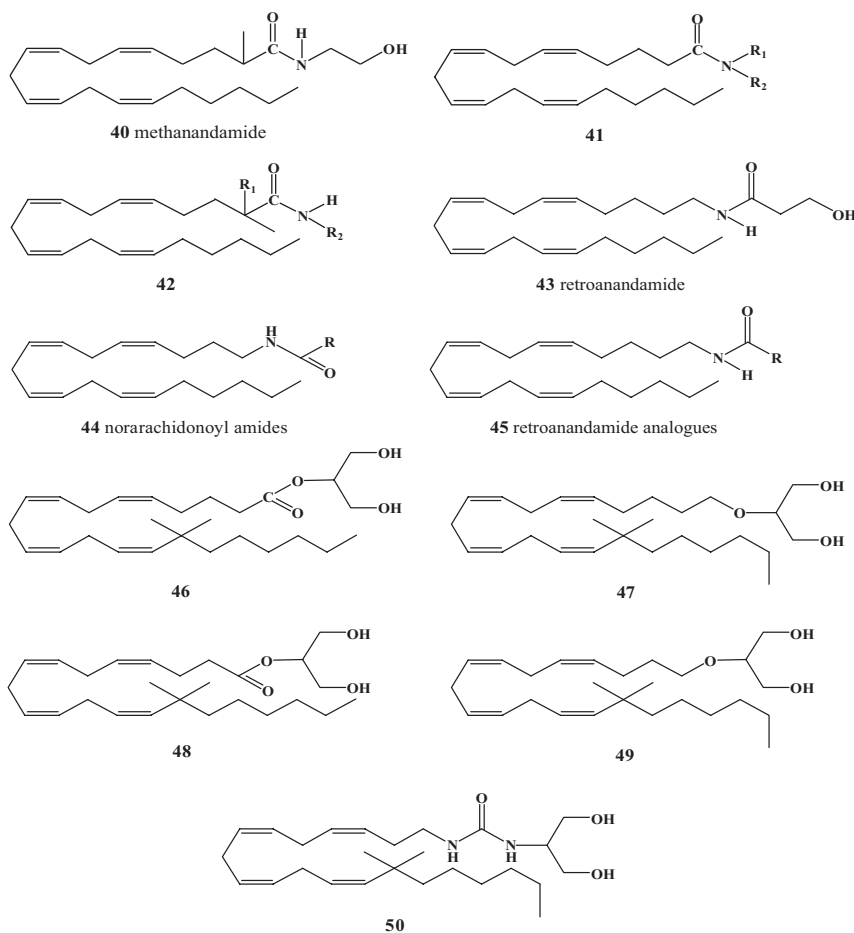
Several years ago a novel nonpsychoactive cannabinoid acid (HU-320) with antiinflammatory properties was synthesized.²⁴⁵

B. Synthetic Endocannabinoid Analogues

(R)-methanandamide (AM-356, **40**), is a chiral analog of the endocannabinoid ligand anandamide. It is more stable to hydrolysis by FAAH than anandamide, as the methyl group adjacent to the amide moiety interferes with this enzyme. Moreover, it is 40-fold more selective for the CB₁ than the CB₂ receptors, with K_i values of 20 ± 1.6 nM for the CB₁ receptor,²⁴⁶ and 815 nM CB₂ receptor.²⁴⁷ In order to establish the structural requirements for binding to the CB₁, Sheskin et al.²⁴⁸ performed an SAR analysis of numerous fatty acid amides and ethanolamides. In the 20: 4, n-6 series, N-monoalkylation, up to a branched propyl group, leads to significant binding (K_i values of 11.7 ± 2.1 to 60.0 ± 7.4 nM; **41**). α-Monomethylation or α,α-dimethylation of N-propyl derivatives potentiates binding and leads to the most active compounds (K_i values of 6.9 ± 0.7 to 8.4 ± 1.1 nM; **42**). The presence of a chiral center on the N-alkyl substituent may lead to enantiomers which differ in their levels of binding.

Retroanandamide (**43**), norarachidonoyl amides (**44**) and retroanandamide analogues (**45**) were partial agonists for the both cannabinoid receptors.²⁴⁹ The ability of dimethylheptyl analogues of 2-AG and noladin ether (**46–50**) to activate the CB₁ receptor were not improved by replacement of the terminal pentyl chain with the dimethylheptyl one.²⁵⁰

To improve enzymatic stability of 2-AG, α-methylated analogues (**51a**, **51b**) of this endocannabinoid were synthesized.²⁵¹ These were slightly weaker CB₁ agonists, but more stable

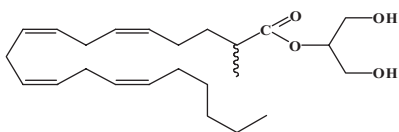


to degradation than 2-AG. The replacement of hydroxyl group by fluorine (**52a**, **52b**, **53**) did not improve the CB₁ activity. New synthetic substances were screened as DAGL α and MAGL inhibitors.²⁵² The novel compound, UP-101 (**54**) inhibited both DAGL α and MAGL with similar potencies. As the most potent inhibitors of DAGL α were O-3,640 (**55**), and O-384 (**56**).

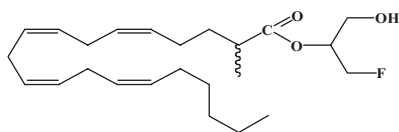
It was found recently,²⁵³ that alkylamides A1 (**57**) and A2 (**58**) from *Echinacea* bind to the CB₂ receptor more strongly (the K_i values are for CB₂ receptor 57 and 60 nM, for CB₁ receptor 6.21 and 1.94 μ M) than the endocannabinoid anandamide (for CB₂ receptor 218 nM and for CB₁ receptor 37 nM). All these compounds significantly inhibited lipopolysaccharide-induced tumor necrosis factor α , IL-1 β , and IL-12p70 expression in a CB₂-dependent manner.

Urbani et al.²⁵⁴ synthesized 35 novel fatty acid amides. Three amino groups improved the metabolic stability of arachidonoylamides. Some of these compounds had moderate affinity to CB₁ receptors and up to 20-fold CB₁/CB₂ selectivity—UP28 (**59**) (CB₁ 200 nM, CB₂ 4.0 μ M), UP30 (**60**) (CB₁ 800 nM, CB₂ > 10 μ M), UP63 (**61**) (CB₁ 200 nM, CB₂ 4.0 μ M), UP66 (**62**) (CB₁ 150 nM, CB₂ 4.3 μ M), UP70 (**63**) (CB₁ 300 nM, CB₂ 5.1 μ M), and UP27-18 (**64**) (CB₁ 500 nM, CB₂ > 10 μ M).

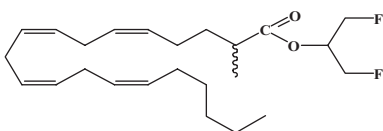
Two new potent and selective anandamide uptake inhibitors as potential antispastic drugs in multiple sclerosis were synthesized.²⁵⁵ These compounds—O-3,246 (**65**) and O-3,262 (**66**)—are structural analogs of previously reported selective inhibitor (O-2,093, **67**) of the reuptake of anandamide.



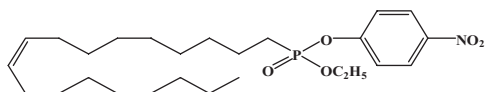
51a (R)
51b (S)



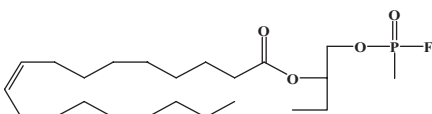
52a (R)
52b (S)



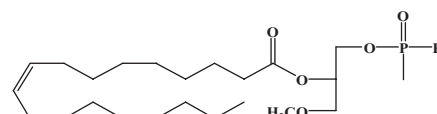
53 (R)



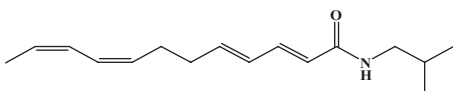
54 UP101



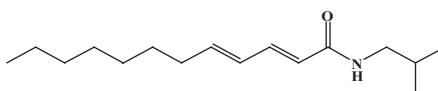
55 O-3640



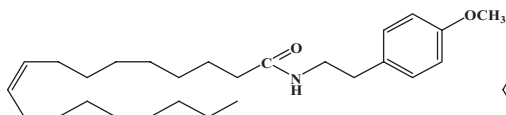
56 O-3841



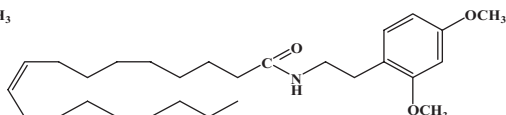
57 A1



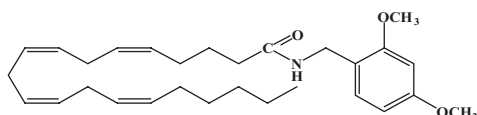
58 A2



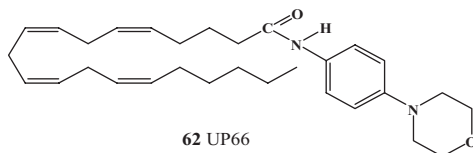
59 UP28



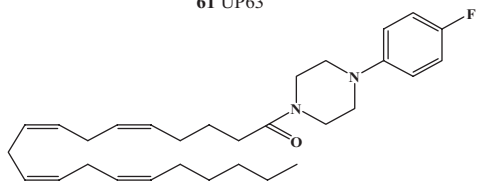
60 UP30



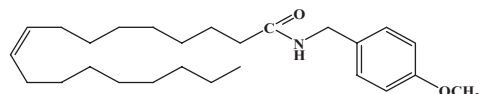
61 UP63



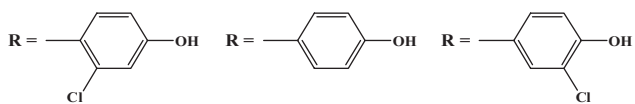
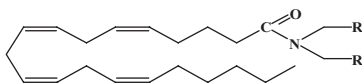
62 UP66



63 UP70



64 UP27-18

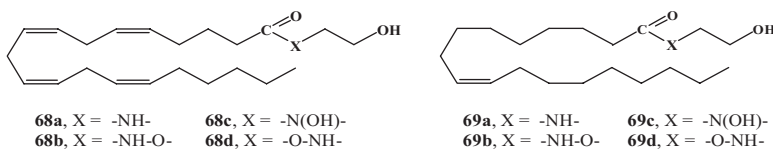


65 O-3246

66 O-3262

67 O-2093

The three amide oxyhomologues of the anandamide (**68a–d**), oleoylethanolamide (**69a–d**) and palmitoylethanolamide were prepared.²⁵⁶ The compound **68b** is the first known compound of this type, which showed better affinity to CB₂ receptor (K_i for CB₁ = 470 nM, for CB₂ = 81 nM).

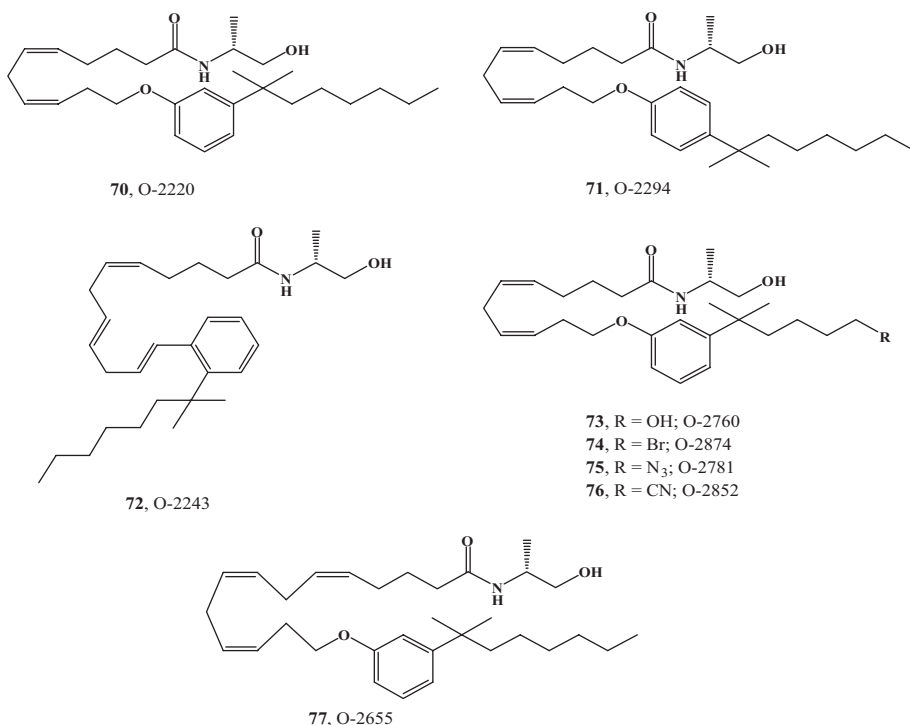


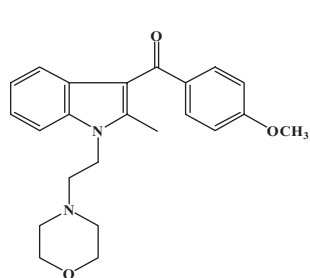
The end pentyl side chain of anandamide, which is similar to that of the Δ^9 -THC side chain was substituted with dimethylheptyl (DMH) one. As DMH side chain increased potency of this compound, it was expected that it will be similar with such anandamide analogs. Bourne et al.²⁵⁷ designed and developed eight such novel hybrid ligands (**70–77**). Compounds **70**, **74**, **75**, and **76** were found to have very high binding affinity not only to CB₁ receptors, but also to CB₂ ones.

C. Synthetic Noncannabinoid Ligands

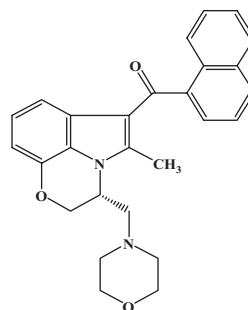
The first noncannabinoids were derivatives of the aminoalkylindole anti-inflammatory, pravadoline (**78**). Not only were these compounds cyclooxygenase inhibitors, but cannabinoid agonists.²⁵⁸ *In vitro* structure-activity relationship studies of these compounds led to numerous new compounds with CB receptor agonist activity, including the conformationally restricted derivative WIN-55212-2 (**79**).^{259–261}

Shortly thereafter, the first potent and selective CB₁ antagonist was created, SR-141716A (**80**).²⁶² Followed by the CB₁ antagonist, LY320135 (**81**), which is not as selective as the previous

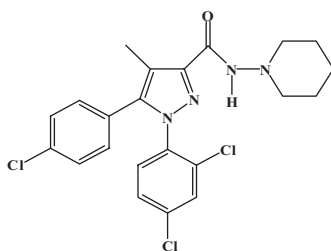
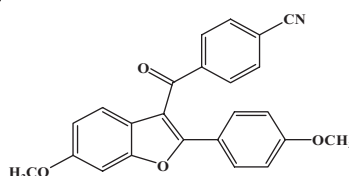




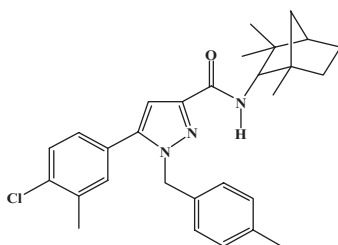
78 Pravadoline



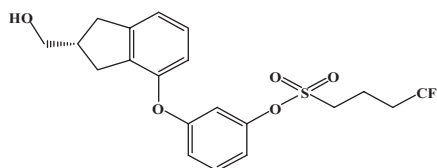
79 WIN 55212-2

80 SR 141716A
(rimonabant)

81 LY320135



82 SR144528



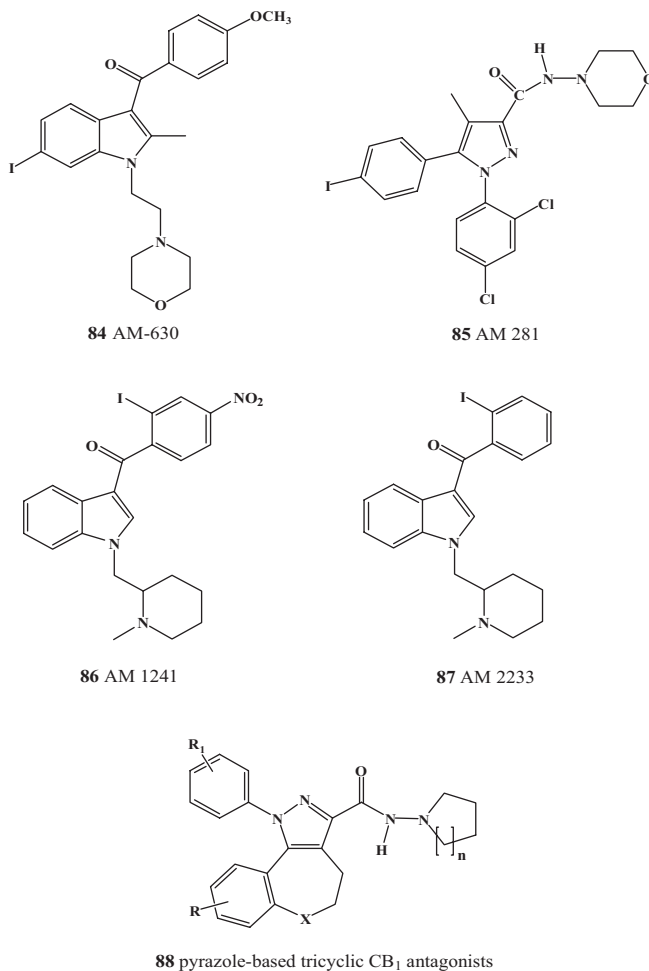
83 BAY 38-7271

one. This substituted benzofuran reverses anandamide-mediated adenylate cyclase inhibition and also blocks WIN-55212-2 mediated inhibition of N-type calcium channels.²⁶³

The Sanofi group also described the first potent and selective antagonist of the peripheral cannabinoid receptor (CB₂), SR 144528 (**82**).^{264,265}

Traumatic brain injury is a major cause of mortality and morbidity. There is no effective drug to treat brain-injured patients. We found that on closed head injury the amounts of 2-AG produced by the brain are increased 10-fold, and that this endocannabinoid apparently has a neuroprotective role, as administration of 2-AG to mice with head trauma reduces both the neurological damage and the edema.²⁶⁶ Numerous other groups have recorded work on various aspects of cannabinoids as neuroprotective agent.²⁶⁷ On this basis a structurally novel, highly potent CB₁/CB₂ cannabinoid receptor agonist, BAY 38-7271 (**83**), was prepared and shown to have pronounced neuroprotective efficacy in a rat traumatic brain injury model.^{268–271}

6-Iodo-pravadoline (AM-630, **84**), an aminoalkylindole, attenuates the ability of a number of cannabinoids to inhibit electrically evoked twitches of the isolated mouse vas deferens.²⁷² AM-630 behaves also as a competitive antagonist of cannabinoid receptor agonists in the guinea pig brain.²⁷³ AM-630 also antagonizes the ability of the cannabinoid agonist WIN 55,212-2 to stimulate guanosine-5'-O-(3-[³⁵S]thio)triphosphate ([³⁵S]GTPγS) binding in mouse brain membrane preparations.²⁷⁴



Gatley et al. have developed a novel radioligand, [¹²³I]AM-281 (**85**), structurally related to the CB₁ selective antagonist SR-141716A, suitable for *in vivo* studies of the central cannabinoid receptor and for imaging this receptor in the living human brain.²⁷⁵

Scientists at the University of Connecticut have synthesized and studied a series of aminoalkylindoles as selective CB₂ agonists. The compounds are stated to be useful for the treatment of pain, glaucoma, multiple sclerosis, and other diseases and disorders. Compound AM1241 (**86**) has a high affinity for the CB₂ receptor in a mouse spleen preparation ($K_i = 3.4 \pm 0.5$ nM), with good selectivity *versus* the CB₁ receptor in a rat brain preparation ($K_i = 280 \pm 41$ nM). This compound has recently been found to inhibit neuropathic pain in rodents.²⁷⁶

AM-2233 (**87**), a novel aminoalkylindole CB₁ agonist, was found to have a potency greater than WIN55212-2 in *in vitro* assays, but has a similar potency in a mouse locomotor assay. It was suggested that its behavioral effects could have been mediated, in part, by action on another receptor type in addition to the CB₁ receptor. AM-2233 represents the first agonist CB₁ receptor ligand ($K_i = 0.4$ nM) with potential as an *in vivo* imaging agent for this receptor.^{277,278} Stoit et al. have reported the syntheses and biological activities of potent pyrazole-based tricyclic CB₁ receptor antagonists (**88**).²⁷⁹ Additional information on cannabinoid receptor agonists and antagonists is reported in Barth's review.²⁸⁰

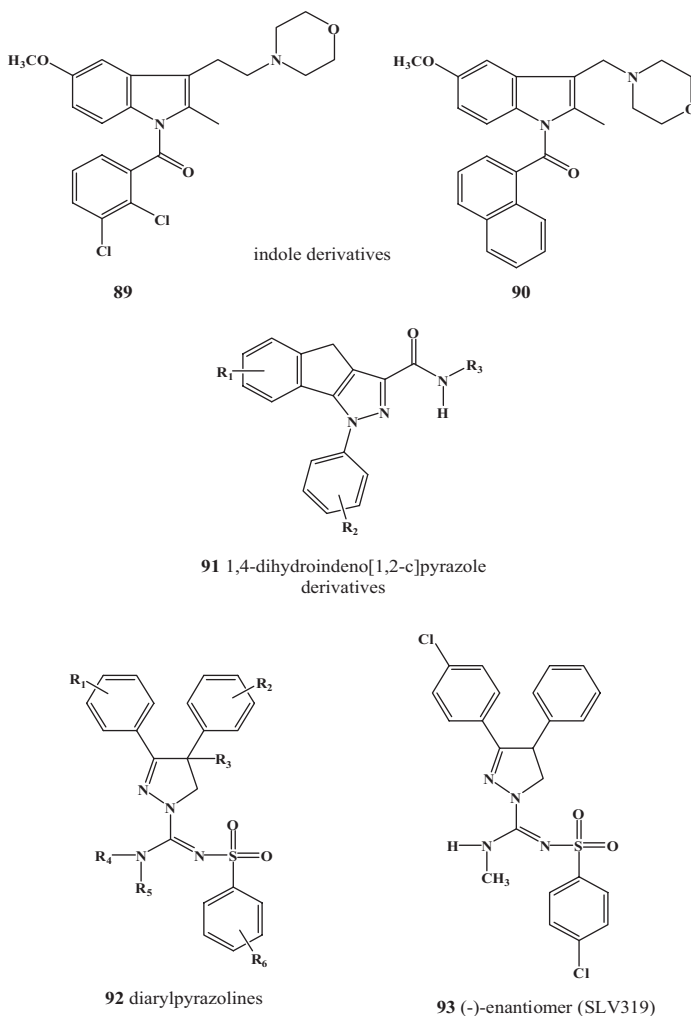
Gallant et al.²⁸¹ have described two indole-derived compounds (**89**, **90**), with binding potency for the human peripheral cannabinoid receptor (hCB₂) in the nanomolar region. They are highly selective.

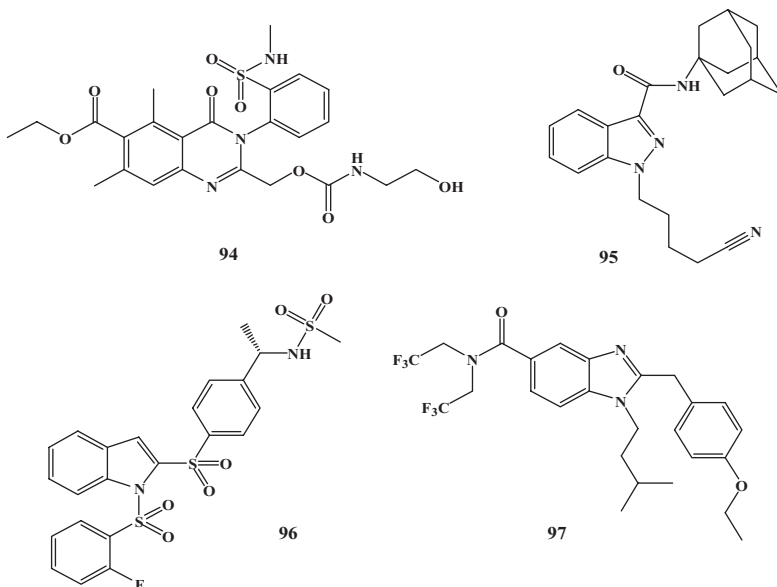
A new series of rigid 1-aryl-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxamides (**91**) was recently designed.²⁸² Seven of the new compounds displayed very high *in vitro* CB₂ binding affinities. Four compounds showed very high selectivity for the CB₂ receptor.

Recently the discovery of a further class of diarylpyrazolines (**92**) with high potency and selectivity for the CB₁ receptor was described.²⁸³ These compounds were found to be CB₁ antagonists. SLV319 (**93**) was found to be a potent CB₁ antagonist ($K_i = 7.8$ nM) close to that of the Sanofi compound SR141716A with more than 1,000-fold selectivity against CB₂.

Additional synthetic compounds, which bind to the CB₁ and/or CB₂ receptors, have been mentioned in patents. These were recently reviewed by Hertzog.²⁸⁴

Novartis AG, has recently filed a patent application on a series of quinazolines as cannabinoid agonists useful for the treatment of pain, osteoarthritis, rheumatoid arthritis and glaucoma, among other indications.²⁸⁵ Compound **94** binds to both CB₁ ($K_i = 34$ nM) and CB₂ ($K_i = 11$ nM). The patent application refers to the compound as having CB₂ agonist activity. Additionally, this





compound has been shown to be active in a rodent neuropathic pain model when administered at a dose of 0.5 mg/kg p.o.

The University of Connecticut has disclosed a series of indazole derivatives that have been found to act as agonists of cannabinoid receptors.²⁸⁶ The compounds exhibit a range of selectivities for CB₂ over CB₁. Compound **95**, for instance, exhibited K_i values of 2.28 and 0.309 nM for the CB₁ and CB₂ receptors, respectively. This compound produced dose-dependent antinociception to thermal stimulus in rats. The compound reduced locomotor activity in rats after intravenous administration, an effect attributed to activation of the CB₁ receptor.

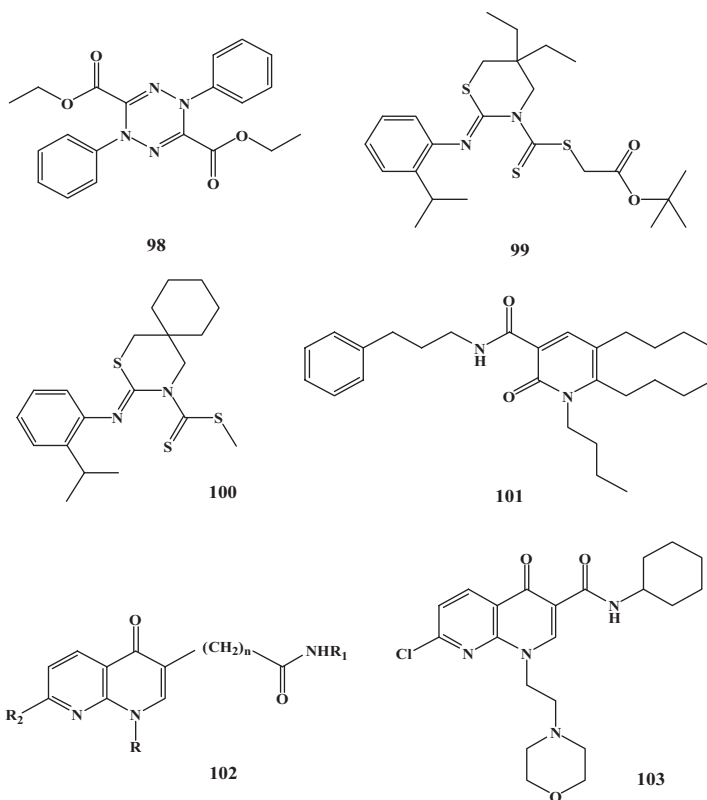
A series of aromatic CB₂ agonists has been disclosed by Schering-Plough Research Institute.^{287,288} The compounds are reported to have anti-inflammatory and immunomodulatory activities, and to be active in cutaneous T cell lymphoma, diabetes mellitus and other indications. Compound **96** is stated to bind to CB₂ with a K_i value in the range 0.1–10 nM.

Researchers at AstraZeneca have disclosed a series of benzimidazoles and azabenzimidazoles to be CB₂ agonists.²⁸⁹ The compounds are described as useful in the treatment of pain, cancer, multiple sclerosis, Parkinson's disease, Huntington's chorea, transplant rejection and Alzheimer's disease. Cannabinoid receptor selectivity data are provided for some of the new compounds. For instance, compound **97** binds to CB₂ (K_i = 3.1 nM) with much greater affinity than to CB₁ (K_i = 2.8 μM). No *in vivo* data are provided for the compounds.

The University of Connecticut has disclosed a series of dihydrotetrazines and derivatives as CB₂ agonists.²⁹⁰ Compound **98** is reported to be a potent CB₂ agonist (K_i = 19 nM) with 88-fold selectivity for the CB₂ over the CB₁ receptor. Such compounds are reported to be useful in the treatment of pain, glaucoma, multiple sclerosis, Parkinson's disease, Alzheimer's disease, and other disorders.

Shionogi has also disclosed two series of thiazine-containing CB₂ agonists, of which compounds **99** and **100** are examples.^{291,292} Selectivity data for several of the compounds with regard to CB₂:CB₁ affinities are described. For example, compound **90** binds to CB₂ with a K_i value of 0.3 nM and a K_i value of >5,000 nM for CB₁. Compound **91** displayed a K_i value of 1.2 nM at the CB₂ receptor and 80 nM at the CB₁ receptor. When dosed orally at 100 mg/kg in a mouse pruritis model, this compound reduced scratching by 98% relative to control animals.

Shionogi has disclosed a series of amide-containing CB₂ modulators stated to be useful in the treatment of inflammation, nephritis, pain, allergies, rheumatoid arthritis, multiple sclerosis, brain



tumors, and glaucoma.²⁹³ Compound **101** was found to bind to the CB₂ receptor with a K_i value of 4 nM, with very little affinity for CB₁ (K_i < 5 μM).

Recently 1,8-naphthyridin-4(1H)-on-3-carboxamide derivatives (**102**) as new ligands of cannabinoid receptors were synthesized.²⁹⁴ Some of these compound possess a greater affinity for the CB₂ receptor than for the CB₁ receptor. Compound 7-chloro-*N*-cyclohexyl-1-(2-morpholin-4-ylethyl)-1,8-naphthyridin-4(1H)-on-3-carboxamide (**103**) revealed a good CB₂ selectivity (CB₁, K_i = 1 μM; CB₂, K_i = 25 ± 1.8 nM).

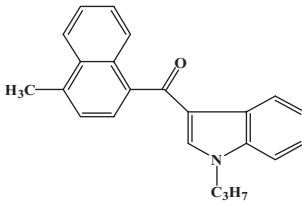
Indole derivatives were prepared and tested for their CB₁ and CB₂ receptor affinities.²⁹⁵ Three new, highly selective CB₂ receptor agonists were identified, namely JWH-120 (**104**) (CB₁, K_i = 1,054 ± 31 nM; CB₂, K_i = 6.1 ± 0.7 nM), JWH-151 (**105**) (CB₁, K_i > 10,000 nM; CB₂, K_i = 30 ± 1.1 nM) and JWH-267 (**106**) (CB₁, K_i = 381 ± 16 nM; CB₂, K_i = 7.2 ± 0.14 nM).

AM251 (**107**), an analog of the cannabinoid receptor antagonist SR141716A, has been shown to be a cannabinoid CB₁ receptor selective antagonist.²⁹⁶

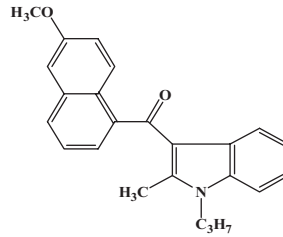
In earlier reports several interesting potent ligands were described. New potent ligands, BML-190 (**108**), L-768242 (**109**), and L-759787 (**110**), for the human peripheral cannabinoid receptor were synthesized.²⁹⁷ Results of New and Wong²⁹⁸ demonstrate that BML-190 and AM251 (cannabinoid antagonist and inverse agonist) behave as inverse agonists at the human CB₂ receptor acting *via* Gα_{i/o} and Gα_q family-coupled pathways.

Hurst et al.²⁹⁹ designed and synthesized an SR141716A analog, called VCHSR (**111**). This ligand acted as a neutral antagonist at wild-type CB₁. Ruiu et al.³⁰⁰ synthesized another putative cannabinoid ligand, antagonist NESS 0327 (**112**), with high selectivity for the cannabinoid CB₁ receptor.

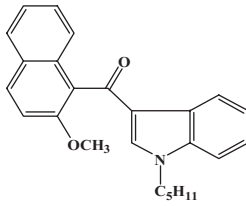
A novel selective ligand, JTE907 (**113**) was reported as an inverse agonist (binding affinity for rat CB₁ was 1.05 μM and for CB₂ 0.38 nM, respectively).³⁰¹ This compound has antiedema effects *in vivo*.



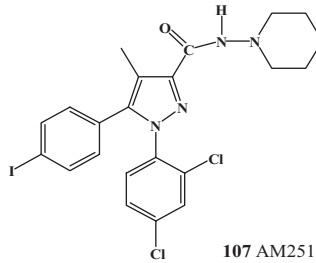
104 JWH-120



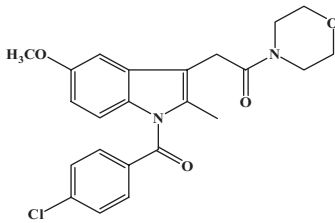
105 JWH-151



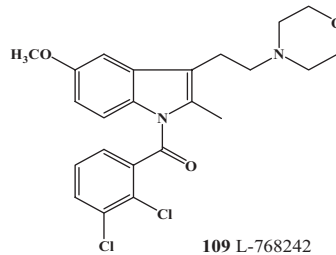
106 JWH-267



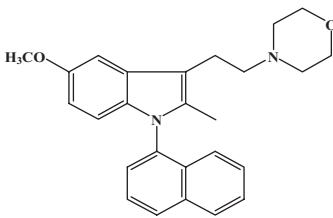
107 AM251



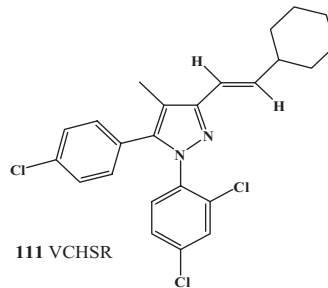
108 BML-190



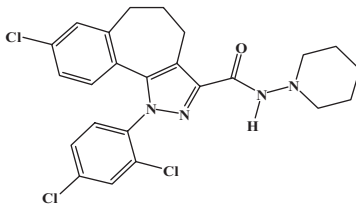
109 L-768242



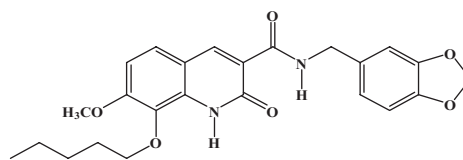
110 L-759787



111 VCHSR

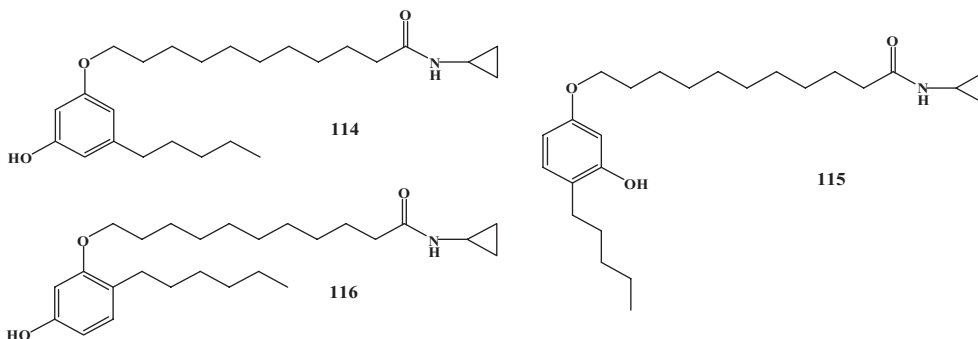


112 NESS 0327

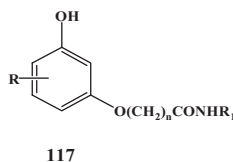


113 JTE907

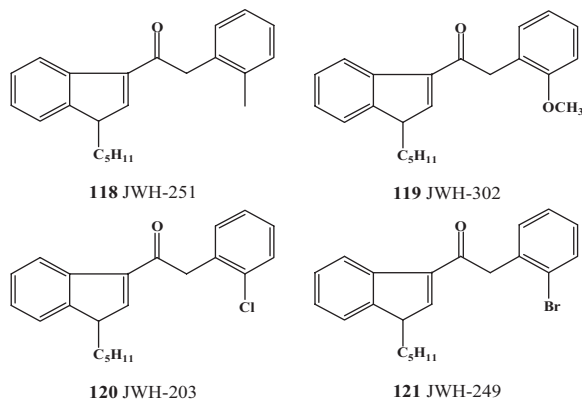
New potent compounds with high affinity to CB₁ and CB₂ receptors were designed, synthesized and biologically evaluated by an Italian scientific group.³⁰² The compounds **114** showed nanomolar affinity for both receptors. The highest selectivity for the CB₂ receptor were shown by compound **115** (a 26-fold selectivity over CB₁ receptors—350 nM and >10 μM) and **116** (30 and 210 nM). These cyclopropylamides are more potent than the respective ethanolamides.



The study of the novel cannabinoid receptor ligands of Brizzi et al.³⁰³ continued over the next few years. New and potent compounds (**117**) were synthesized and tested. The most active compound (R = 4-*n*-hexyl, N = 7, R₁ = *c*-C₃H₅) revealed for the CB₁ receptor, activity of K_i = 56 nM and for the CB₂ receptor, 280 nM.



A new class of cannabimimetic indoles, 1-pentyl-3-phenylacetylindoles, has been prepared and their affinity to the cannabinoid receptors studied.³⁰⁴ Compounds JWH-251 (**118**) and JWH-302 (**119**) were found moderately selective for the CB₁ receptor (29 and 17 nM) and are full agonists at this receptor and partial agonists at the CB₂ receptor (146 nM and 89 nM). Other compounds JWH-203 (**120**) and JWH-249 (**121**) have good affinity for both receptors (8 nM vs. 7 nM, respectively and 8.4 nM vs. 20 nM, respectively).



Affinity of 1-alkyl-2-aryl-4-(1-naphthoyl)pyrroles for central as well as for peripheral cannabinoid receptors was determined by Huffman et al.³⁰⁵ 2-phenylpyrroles (**122**) and 2-arylpyrroles (**123**) were prepared. Most of these compounds had very good affinity to both receptors (single digits or tens of nM).

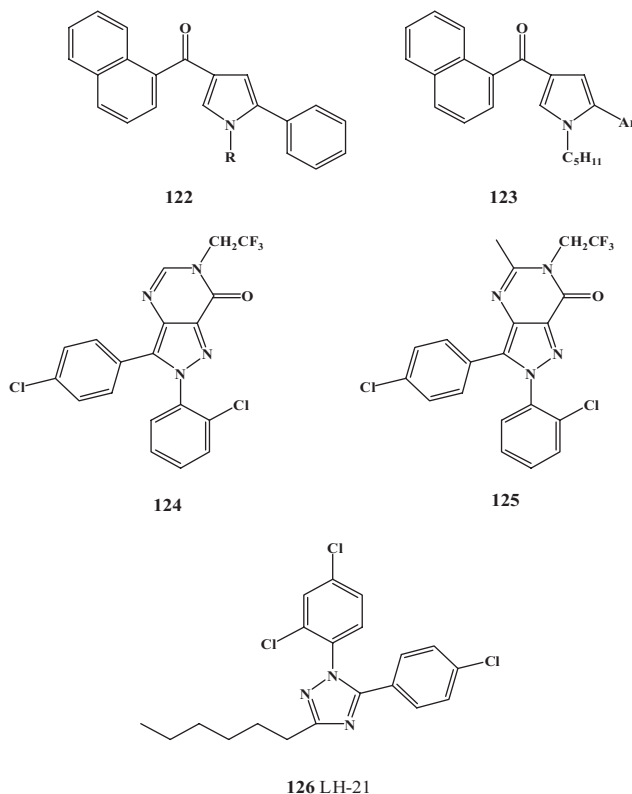
Carpino et al.³⁰⁶ prepared and evaluated a series of conformationally-constrained bicyclic, selective central cannabinoid receptor CB₁ antagonists and inverse agonists. Two of these compounds (**124** and **125**) showed high selective affinities for central cannabinoid receptors ($K_i = 0.3$ nM and 0.6 nM for hCB₁ and for hCB₂; both compounds bind with $K_i > 10$ μM). Both compounds showed good anorectic activities in a fasting-induced refeeding model in rats following oral administration.

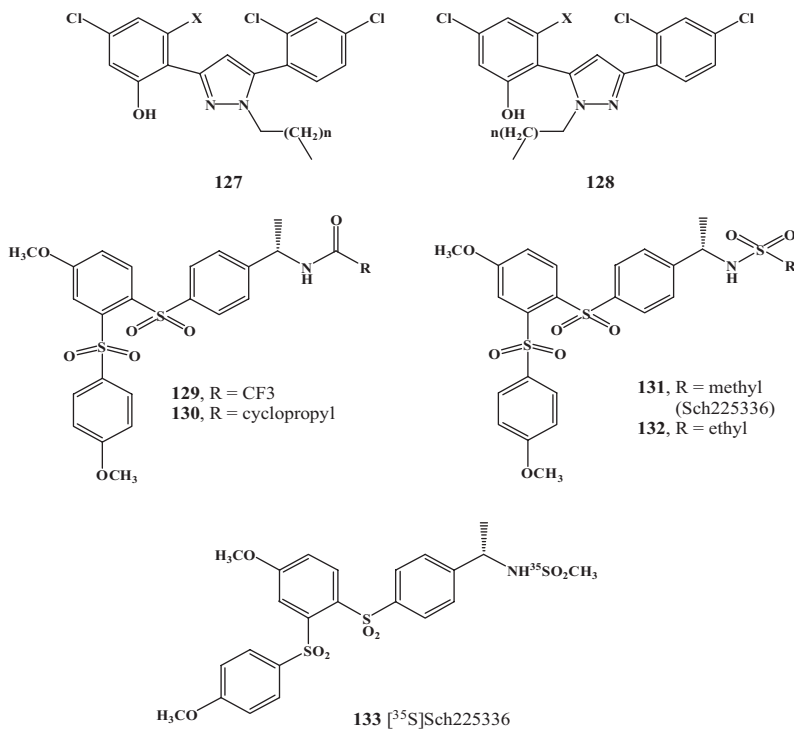
A new series of 1,2,4-triazoles was prepared and identified as a new heterocyclic core with potential cannabinoid properties.³⁰⁷ Compound LH-21 (**126**) behaved as a silent CB₁ antagonist with moderate affinity to CB₁ receptor. This new neutral cannabinoid CB₁ receptor antagonist has clear anti-obesity effects and has poor penetration in the brain, reducing side effects such as motor alterations and anxiety of reference inverse agonists.³⁰⁸

A series of *N*-alkyl-3(5)-phenyl-5(3)-hydroxyphenylpyrazones (**127** and **128**) was synthesized and tested as CB₁ cannabinoid ligands.³⁰⁹ All tested substances displayed only moderate or low CB₁ receptor affinity.

Lavey et al.³¹⁰ described triaryl bis-sulfones as a novel class of specific CB₂-receptor ligands. Four of these compounds (**129**–**132**) with high affinity for the human receptor are such CB₂-selective ligands. Study of the most potent and selective compound, Sch225336 (**131**) (K_i for CB₂ 0.4 nM and for CB₁ 905 nM, respectively), suggest that the class are inverse agonists for the CB₂ receptor.

CB₂-selective radioligand [³⁵S]Sch225336 (**133**) was synthesized and used for detail autoradiographic analysis of CB₂ in lymphoid tissues.³¹¹





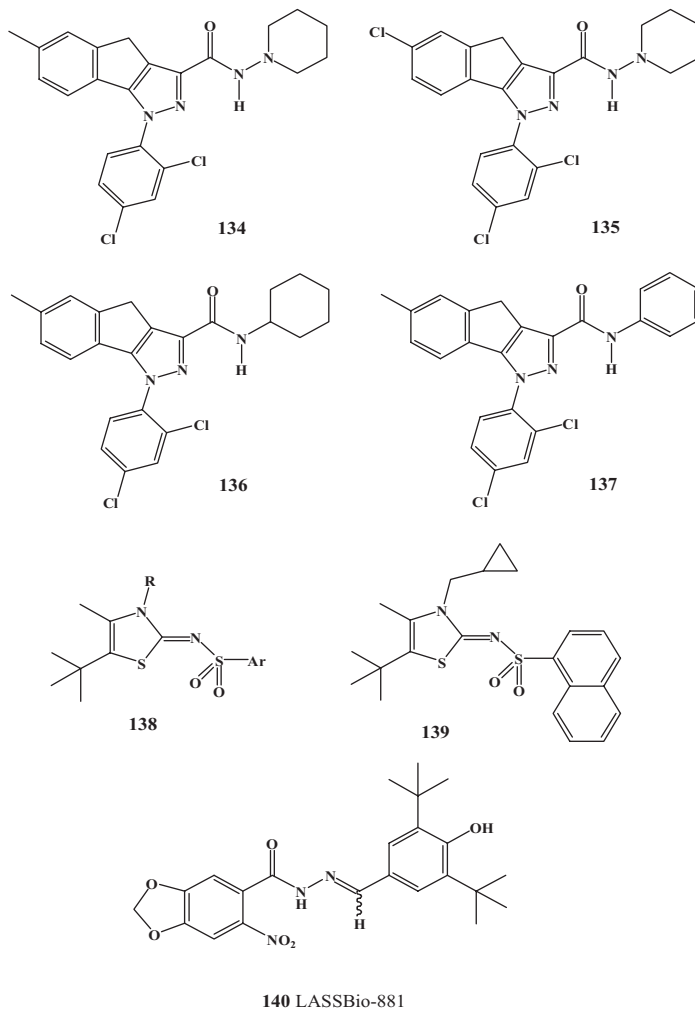
Recently the synthesis and the CB₂-affinity of several new 1,4-dihydroindeno[1,2-*c*]pyrazol-based ligands was reported.³¹² Seven of the new compounds displayed very high *in vitro* CB₂ binding affinities (K_i between 0.34 and 0.9 nM). Compounds **134** and **135** showed the highest selectivity for the CB₂ receptor while compound **136** had the highest affinity *and* selectivity. Another series of analogues of the previously reported CB₂ ligands revealed several compounds with CB₁ affinity in nM range with moderate or negligible affinity towards CB₂ receptors.³¹³ This scientific team prepared a series of new ligands, tricyclic pyrazoles, with high CB₂ selectivity.³¹⁴ Compounds **134**–**137** were shown to be agonists in an *in vitro* model based on human promyelocytic leukemia HL-60 cells. All four compound have high CB₂ selectivity ($K_i = 37$ pM, 340 pM, 7.6 nM, and 32.8 nM) against CB₁ affinity ($K_i = 363$ nM, 2.05 μ M, 900 nM, and 2,409 μ M).

Recently the identification of novel, selective, and potent CB₂ receptor agonists was reported.³¹⁵ A novel series of sulfonamide derivatives (**138**) was synthesized and evaluated. The presence of a 1-Naphthyl group (**139**) resulted in a significant increase in the affinity for the CB₂ receptor (CB₂ IC₅₀ = 16 nM, CB₁ IC₅₀ = 1.7 μ M). This compound was found to be a full CB₂ receptor agonist.

Last year saw the report of a new class of ligands,³¹⁶ 6-nitro-3,4-methylenedioxyphenyl-*N*-acylhydrazone derivatives. One of them, LASSBio-881 (**140**), binds to the CB₁ receptor and was identified as a novel central antinociceptive and peripheral anti-inflammatory compound, which exhibits important antioxidant properties and inhibits T-cell proliferation.

Seventeen new conformationally restricted lipopeptides have been designed, synthesized and evaluated for the CB₁ binding affinity.³¹⁷ Four of these compounds (**141**–**144**) were able to cause at least 50% displacement of radioligand from CB₁ receptors (K_i for these four particular compounds found 42, 37.5, 39, and 20 μ M).

Recently, Horswill et al.³¹⁸ provided evidence for allosteric antagonism of the CB₁ receptor by a novel synthetic compound PSNCBAM-1 (**145**). This compound is active *in vivo* in an acute rat feeding model offering prospects as a novel treatment for obesity. In competition binding experiments this compound indicated positive modulation of agonist binding.

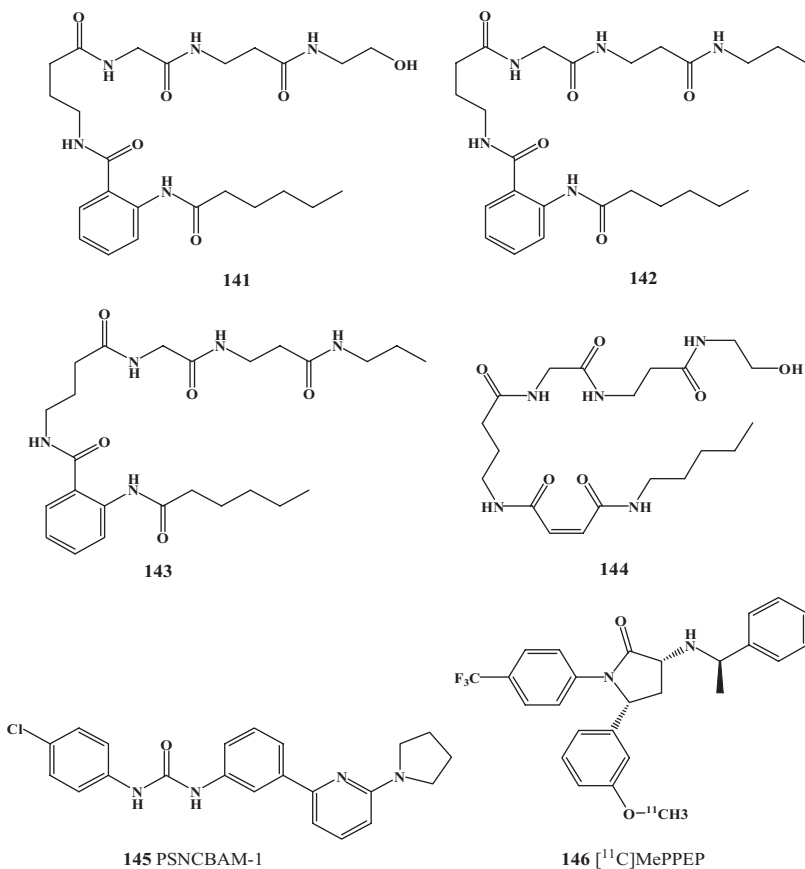


A CB₁ receptor neutral antagonist AM4113 (*N*-piperidin-1-yl-2,4-dichlorophenyl-1H-pyrazole-3-carboxamide analog) could be useful as appetite suppressant. In the same dose range that suppressed feeding did not induce conditioned gaping in rats. This compound does not show inverse agonist properties. It was shown that this compound shares the appetite suppressant and weight loss effects of inverse agonists. If this compounds display similar properties in humans, it could be developed into a new anti-obesity agent.^{319–321}

A new positron emission tomography radioligand [¹¹C]MePPEP (**146**) with high affinity and selectivity for CB₁ receptor ($K_b = 0.574 \pm 0.207$ nM) compared to CB₂ = 363 ± 87.4 nM, which is a CB₁ mixed inverse agonist and antagonist, was used to image CB₁ receptors in monkey brain. The uptake of [¹¹C]MePPEP in nonhuman primate brain demonstrated reversible labeling of CB₁ receptors. These promising results justify trials of this tracer in humans.³²²

9. (ENDO)CANNABINOIDS IN THERAPY

Extensive information about Cannabis as a medicament goes beyond the possibilities of this article and we can refer readers to several comprehensive books^{323–327} and recent reviews^{328,329} on this subject.



The therapeutic importance of the endocannabinoid system is today without any doubt well recognized. This subject has appeared recently in books³³⁰ and in numerous reviews.^{331–344}

Nausea and vomiting are common side effects of chemotherapy treatment for cancer but not everyone experiences nausea and vomiting during chemotherapy. Patients usually do not experience nausea and/or vomiting before or during chemotherapy until after they have received several courses of treatment. Then chemotherapy will often be accompanied by severe nausea and vomiting. The endocannabinoid 2-AG is a potent emetogenic agent and its emetic effects can be blocked by diverse cannabinoid agonists as well as the CB₁ receptor antagonist SR141716A. The emetic action of the endocannabinoid 2-AG and the antiemetic effects of well established cannabinoids (Δ^9 -THC, nabilone and levonantradol) are mediated *via* cannabinoid CB₁ receptors. It is clear that these cannabinoids possess significant antiemetic properties in patients receiving chemotherapy.³⁴⁵ Δ^9 -THC was first used to control nausea and vomiting during chemotherapy in the 1970s. Side effects of nabilone and levonantradol, synthetic cannabinoids, which are also effective antiemetic are similar to THC and are mild.³⁴⁶ When Δ^8 -THC was administered to children, treated with different antineoplastic drugs, vomiting was completely prevented. The observed side effects were negligible.³⁴⁷ These results have already been reviewed.³⁴⁸

The physiological control of appetite and satiety, in which numerous neurotransmitters and neuropeptides play a role, is extremely complex. A considerable research has examined endocannabinoid involvement in appetite, eating behavior and body weight regulation.^{349,350} It is now confirmed that endocannabinoids, acting at brain CB₁ cannabinoid receptors, stimulate appetite and ingestive behaviors. There is strong evidence of an endocannabinoid role in energy metabolism

and fuel storage. Recent developments point to potential clinical benefits of cannabinoid receptor antagonists in the management of obesity, and of agonists in the treatment of other disorders of eating and body weight regulation.³⁵¹ The presence of endocannabinoids in the developing brain and in maternal milk have led to evidence for a critical role for CB₁ receptors in oral motor control of suckling during neonatal development. Therapeutically, appetite stimulation by cannabinoids has been studied for several decades, particularly in relation to cachexia and malnutrition associated with cancer, acquired immunodeficiency syndrome, or anorexia nervosa. The exciting progress in the understanding of how the endocannabinoid/cannabinoid receptor systems influence appetite and body weight is stimulating the development of therapeutic orexigenic and anorectic agents. The role of cannabinoid CB₁ receptor activation for milk suckling in newborns may open new doors toward understanding nonorganic failure-to-thrive in infants, who display growth failure without known organic cause.³⁵² Individuals with HIV constitute the largest group using cannabinoids for medicinal reasons. For HIV-positive marijuana smokers, both dronabinol and marijuana are well tolerated and produce substantial and comparable increases in food intake.³⁵³ The pathogenesis of AIDS-associated anorexia involves any one or a combination of several factors, including malnutrition and nutrient abnormalities, gastrointestinal dysfunction, metabolic dysfunctions, neuropsychiatric disturbances, economic and sociocultural factors, and anorexigenic medications. Two pharmacologic agents, the cannabinoid dronabinol and the synthetic progesterone megestrol acetate, have been studied in a double-blind appetite stimulation study. The study focused on appetite stimulation and weight gain as end points in patients with AIDS-related weight loss.³⁵⁴ More than 60% of advanced cancer patients suffer from anorexia and cachexia. Cannabinoids stimulate appetite and food intake. Further study of cancer-cachexia pathophysiology and the role of endocannabinoids will help to develop cannabinoids without psychotropic properties, which will help cancer patients suffering from cachexia and improve outcomes of clinical antitumor therapy.³⁵⁵ The endocannabinoid system regulates the rewarding properties of food by acting at specific mesolimbic areas in the brain. Drugs that interfere with this system, especially CB₁ receptor antagonists, should be considered as useful adjuncts to lifestyle and behavior modifications in the treatment of obesity.³⁵⁶

Cannabinoid CB₁ receptors are involved in ocular physiology and may regulate intraocular pressure. Anandamide, 2-AG, and the anandamide congener, palmitoylethanolamide, were detected in all the human tissues examined. In eyes from patients with glaucoma, significantly decreased 2-AG and PEA levels were detected in the ciliary body. The findings suggest that these endogenous compounds may have a role in this disease, particularly with respect to regulation of IOP.³⁵⁷ Cannabinoids may reveal themselves to be useful for the treatment of glaucoma in a quite comprehensive manner: lowering intraocular pressure, restoring microcirculation, inhibiting apoptosis, and minimizing free radical damage. This hypothetical multiple mechanism may help to explain why the use of marijuana has preserved the sight of those unresponsive to other glaucoma therapies.³⁵⁸

A reviews of clinical and preclinical evidence that supports the use of cannabinoid receptor agonists for the management of multiple sclerosis was published recently. Evidence that the endocannabinoid system plays a protective role in multiple sclerosis was also discussed.^{359–363} Cannabinoid CB₁ and/or CB₂ receptor activation may suppress some of the pathological changes that give rise to signs and symptoms of multiple sclerosis. There is increasing evidence to suggest that Cannabis can ameliorate muscle-spasticity in multiple sclerosis, as was objectively shown in experimental autoimmune encephalomyelitis models.³⁶⁴ The high level of CNS IFN- γ associated with experimental autoimmune encephalomyelitis disrupts endocannabinoids-mediated neuroprotection while maintaining functional cannabinoid receptors. These observations provide additional support for the use of cannabinoid-based medicine to treat multiple sclerosis.³⁶⁵

Posttraumatic Stress Disorder is an anxiety disorder that can develop after exposure to a terrifying event or ordeal in which grave physical harm occurred or was threatened. PTSD was first brought to public attention in relation to war veterans, but it can result from a variety of traumatic

incidents. The “recovery” is actually a case of forgetting what a person remembered.³⁶⁶ The roles of cannabinoid receptors in learning and memory have been well documented using rodents in various memory tests. The endocannabinoid system is required in the acquisition and/or extinction of memory. A hope has been fuelled that the endocannabinoid system might be a valuable therapeutic target for the treatment of these disorders.³⁶⁷ Elevating brain levels of anandamide through either genetic deletion or pharmacological inhibition of its primary catabolic enzyme fatty-acid amide hydrolase (FAAH) potentiate extinction in a fixed platform water maze task. Results of Varvel et al.³⁶⁸ indicate that endogenous anandamide plays a facilitatory role in memory extinction through a CB₁ receptor mechanism of action. Retrieved contextual fear memory is vulnerable to amnesic treatments and the amygdala is critically involved. Cholinergic and histaminergic systems seem to modulate only consolidation, whereas cannabinoids are involved in both consolidation and reactivation. The lability of retrieved memory affords opportunities to treat disorders such as phobias, post-traumatic stress, or chronic pain, and these conditions may help searching for appropriate therapeutic targets.³⁶⁹

Dysfunctional responding to stress is a component of several human neuropsychiatric disorders, including anxiety and panic disorders, post-traumatic stress disorders, premenstrual dysphoria, and quite possibly drug abuse. Endocannabinoids and cannabinoid receptor are involved in the responses of animals to acute, repeated and variable stress. It is very possible that either inhibition or potentiation of endocannabinoid signaling will be an efficacious novel therapeutic approach to one or more human psychiatric diseases.³⁷⁰ The absence of CB₁ receptors reduces the neuroendocrine response and does not affect the behavioral response to moderate stress. Finally, the CB₁ receptor plays a role in modulating the stress response from an early age. These observations suggest that CB₁ receptors participate in the mediation of the stress response and that the absence of these receptors results in a greater vulnerability to stress.³⁷¹ The results of Chhatwal et al.³⁷² demonstrate that the endocannabinoid system can be modulated to enhance emotional learning, and suggest that endocannabinoid modulators may be therapeutically useful as adjuncts for exposure-based psychotherapies such as those used to treat post-traumatic stress disorder and other anxiety disorders.

Cannabinoids suppress behavioral responses to noxious stimulation and suppress nociceptive transmission through activation of CB₁ and CB₂ receptor subtypes. Guindon and Hohmann³⁷³ reviewed behavioral, neurochemical and electrophysiological data, which identify cannabinoid CB₂ receptors as a therapeutic target for treating pathological pain states with limited centrally, mediated side effects. They also discussed therapeutic potential and possible limitations of CB₂-based pharmacotherapies for pathological pain states induced by tissue and nerve injury. Cannabinoid receptor agonists reduce the abnormal pain sensations associated with animal models of neuropathic pain states and they also produce CB₁ receptor mediated side effects. Vuong et al.³⁷⁴ examined the effect of *N*-arachidonyl-glycine in a rat model of neuropathic pain. Their findings suggest that *N*-arachidonyl-glycine may provide a novel analgesic approach to alleviate neuropathic pain.

Nabilone has been approved to treat chemotherapy-induced nausea and vomiting. Recent studies have explored this compound in pain management. Nabilone is ineffective in acute pain but benefits in neuropathic pain and central hypersensitization. Recent guidelines place nabilone as a second to fourth line drug for neuropathic pain.³⁷⁵

Guy and Costa³⁷⁶ invented the use of cannabidiol type compounds or derivatives thereof in the manufacturing of a medicament for the treatment of neuropathic pain.

It is well known that cannabinoid receptor agonists produce relief of pain in a variety of animal models by interacting with cannabinoid receptors. Cheng and Hitchcock³⁷⁷ reviewed the present development of cannabinoid agonists with an emphasis on selective CB₂ agonists and peripherally restricted CB₁ or CB₁/CB₂ dual agonists for treatment of inflammatory and neuropathic pain.

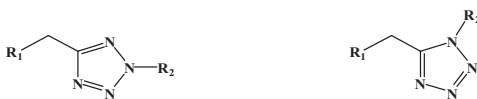
Guy et al.³⁷⁸ invented the use of a combination of cannabidiol and Δ^9 -tetrahydrocannabinol in the treatment of neuropathic pain, in particular peripheral neuropathic pain.

The effects of the synthetic cannabinoid WIN 55,212-2 were examined in a rodent model for neuropathic pain. Their results provide a neural basis for reports of potent suppression by cannabinoids of the abnormal sensory responses that result from nerve injury.³⁷⁹

Cannabinoids are already in legal use in medicine for the prevention of nausea and vomiting caused by cancer chemotherapy and as appetite stimulant in AIDS patients. Two oral formulations, dronabinol (Marinol) and nabilone (Cesamet), are approved by the US Food and Drug Administration for use in chemotherapy-induced nausea and vomiting refractory to conventional anti-emetic therapy. In a number of comparative clinical trials, patients have expressed a clear preference for the cannabinoid, choosing its efficacy over any undesired effects.³⁸⁰ Marinol capsules are also used and approved by the FDA as an appetite stimulant to correct the weight loss related to anorexia in AIDS patients.³⁸¹

10. ADDITION TO BIOSYNTHESIS AND DEGRADATION OF ENDOCANNABINOIDS

Most recently, a new series of tetrazoles, selective and nonfatty acid-based anandamide uptake inhibitors, have been synthesized.³⁸² Some of them, compounds **147** (IC₅₀ 2.3 μM), **148** (IC₅₀ 5.1 μM), **149** (IC₅₀ 4.9 μM), **150** (IC₅₀ 5.0 μM), and **151** (IC₅₀ 5.1 μM), inhibit anandamide cellular uptake process with a relative high potency. Such compounds could contribute to potential therapy of different diseases.



147, R₁ = 4-biphenyl-4-carboxamido;

R₂ = CH₂CON(CH₃)₂

148, R₁ = 4-biphenyl; R₂ = CH₂CN

149, R₁ = 4-biphenyl; R₂ = CH₂CO₂CH₃

150, R₁ = 4-biphenyl; R₂ = CH₂COCH₃

151, R₁ = 4-biphenyl; R₂ = CH₂CON(CH₃)₂

11. FINAL REMARKS

In the near future, the development of new drugs will be concerned with neuroprotection,³⁸³ with neuropathic pain, and the suppression of memory triggering post-traumatic stress disorder.

Anandamide was discovered 16 years ago, and it still has never been administered to a human—but this field has been studied intensively from many points of view. Investigations of anandamide may lead to promising treatments. Researchers have tried to separate the positive effects of marijuana from its negative ones, and the future of Cannabis as a therapeutic agent remains uncertain. Scientists speculate, however, that the brain's natural anandamide system could hold the same medicinal benefits as marijuana and will be easier to decipher and harness for new treatments.

REFERENCES

1. Hanuš L. Personal observations, 2006.
2. Klíma B, Dolní Věstonice. Praha: Nakladatelství Československé akademie věd; 1963.
3. Klíma B, Dolní Věstonice. Praha: Academia; 1983.
4. Soffer O, Adovasio JM, Hyland DC, Klíma B, Svoboda J. Perishable technologies and the genesis of the Eastern Gravettian. *Anthropologie* 1998;36:43–68.
5. Adovasio JM, Soffer O, Hyland DC, Klíma B, Svoboda J. Textil, košíkářství a sítě v mladém paleolitu Moravy. (Textiles, Basketry, and Nets in Upper Paleolithic Moravia.) *Archeol Rozhledy* 1999;51:58–94.

6. Adovasio JM, Soffer O, Hyland DC, Illingsworth JS, Klíma B, Svoboda J. Perishable industries from Dolní Věstonice I: New insights into the nature and origin of the Gravettian. *Archaeol Ethnol Anthropol Eurasia* 2001;2:48–65.
7. Soffer O, Adovasio JM, Hyland DC. Perishable technologies and invisible people: Nets, baskets, and “venus” wear ca. 26,000 B.P. In: Purdy BA, editor. *Enduring records: The environmental and cultural heritage of wetlands*. Oxford: Oxbow Books; 2001. pp. 233–245.
8. Soffer O, Adovasio JM, Hyland DC. The “venus” figurines: Textiles, basketry, gender, and status in the upper paleolithic. *Curr Anthropol* 2000;41:511–537.
9. Mason SLR, Hather JG, Hillman GC. Preliminary investigation of the plant MACRO-remains from Dolní-Věstonice-II and its implications for the role of plant foods in paleolithic and mesolithic Europe. *Antiquity* 1994;68:48–57.
10. Barber EJW. *Prehistoric textiles*. Princeton: Princeton University Press; 1991. p. 508.
11. Hald M. The nettle as a culture plant. *Folk-Liv* 1942;6:28–49.
12. Adovasio JM. personal private communication to L. Hanuš (December 15, 2006).
13. Pringle H. Archaeology: Ice age communities may be earliest known net hunters. *Science* 1997;277:1203–1204.
14. Chang K. *The archaeology of ancient China*. New Haven: Yale University Press; 1968. pp. 111–112.
15. Kung CT. *Archeology in China*. Toronto: University of Toronto Press; 1959; Vol. 1. p. 131.
16. Li H-L. 1974; An archeological and historical account of Cannabis in China. *Econ Bot* 1974;28:437–448.
17. Unschuld PU. *Medicine in China: A history of pharmaceuticals*. Berkeley: University of California Press; 1986. p. 367.
18. Campbell Thompson R. *A Dictionary of Assyrian Botany*. London: The British Academy; 1949. p. 405.
19. Waterman L. *Royal correspondence of the Assyrian Empire*. Letter 368. Ann Arbor: University of Michigan Press; 1930.
20. *The papyrus Ebers. The greatest Egyptian Medical document*. Translated by Bendix Ebbell. Copenhagen: Levin & Munksgaard; 1937, 135 pages.
21. Bryan CP. *Ancient Egyptian medicine: The Papyrus Ebers*. London: Geoffrey Bles; 1930. p. 167.
22. Ghalioungui P. *The Ebers papyrus. A new English translation, commentaries and glossaries*. Cairo: Academy of Scientific Research and Technology; 1987.
23. Scholl R. *Der Papyrus Ebers. Die größte Buchrolle zur Heilkunde Altägyptens*. Leipzig: Schriften aus der Universitätsbibliothek; 2002. p. 7.
24. Manniche L. *An Ancient Egyptian Herbal*. London: British Museum Publications Ltd; 1989. 176 p.
25. *Sacred Books of the East*. Translated by Maurice Bloomfield. Volume 42, Hymns of the Atharva-Veda. Oxford: The Clarendon Press; 1897, 792 pages.
26. *The Vendidad (The Zend-Avesta, part I, Fargard 15, II, 14(43))* (1880) translated by James Darmesteter, *Sacred books of the East, volume 4.*, Oxford University Press; 1880.
27. *Khorda Avesta (Book of Common Prayer)*. Translation by James Darmesteter In: *Sacred Books of the East, American Edition*; 1898.
28. *Book of Arda Viraf*. In: Horne CF, editor. *The Sacred Books and Early Literature of the East, Volume VII: Ancient Persia*. New York: Parke, Austin, and Lipscomb; 1917. p. 316.
29. Avalon A. *Tantra of the great liberation—Mahanirvana Tantra*. New York: Dover publications; 1972. p. 359.
30. Pliny the Elder. *The natural history*. London: Taylor and Francis; 1855.
31. Herodotus. *The history of herodotus. Book 4: Melpomene [70]*. London: Macmillan; 1890.
32. Gunther RT. *The greek herbal of dioscorides*. New York: Hafner Publishing Co.; 1959. p. 701.
33. Dioscorides Pedanius. *De Materia Medica*. Johannesburg: IBIDIS Press; 2000. p. 836.
34. Diodorus Siculus. *The library of history*. London: William Heinemann; 1933.
35. Benetowa S. *Konopie w wierzeniach i zwyczajach ludowych (Hemp in folk belief and customs)*. Warszawa: Instytutu Nauk Antropologicznych i; Etnologicznych Towarzystwa Naukowego Warszawskiego; 1936. p. 22.
36. Benetowa S. Tracing one word through different languages. In: Andrews G, editor. *The book of grass: An anthology on Indian Hemp*. New York: Grove Press; 1967. p. 242.
37. Meissner B. *Babylonien und Assyrien. II: 84*. Heidelberg: Carl Winters Universitätsbuchhandlung; 1925.
38. Klein S. *Tod und Begrabnis in Palestina*. Berlin: H. Itzkowski; 1908.
39. Benet S. Early diffusion and folk uses of hemp. In: Rubin V, editor. *Cannabis and culture*. The Hague: Mouton; 1975. pp. 39–49.
40. Rabin C. Rice in the bible. *J Semit Stud* 1966;11:2–9.
41. Green J. *Cannabis*. New York: Thunder’s Mouth Press; 2002. p. 256.

42. Abel EL. Marijuana—The first twelve thousand years. New York: Plenum Press; 1980. p. 289.
43. ElSohly MA, Slade D. Chemical constituents of marijuana: The complex mixture of natural cannabinoids. *Life Sci* 2005;78:539–548.
44. Wood TB, Spivey WTN, Easterfield TH. Cannabinol. Part I. *J Chem Soc* 1899;75:20–36.
45. Cahn RS. Cannabis indica resin, Part III. The constitution of Cannabinol. *J Chem Soc* 1932; 1342–1353.
46. Jacob A, Todd AR. Cannabis indica. Part II. Isolation of Cannabidiol from Egyptian Hashish. Observations on the Structure of Cannabinol. *J Chem Soc* 1940; 649–653.
47. Ghosh R, Todd AR, Wilkinson S. Cannabis indica, Part V. The synthesis of cannabinol. *J Chem Soc* 1940; 1393–1396.
48. Adams R, Baker BR, Wearn RB. Structure of Cannabinol. III. Synthesis of Cannabinol,1-Hydroxy-3-n-amy-6,6,9-trimethyl-6-dibenzopyran. *J Am Chem Soc* 1940;62:2204–2207.
49. Adams R, Wolff H, Cain CK, Clark JH. Structure of Cannabidiol. V. Position of the alicyclic double bonds. *J Am Chem Soc* 1940;62:2215–2219.
50. Adams R, Loewe S, Pease DC, Cain CK, Wearn RB, Baker BR, Wolff H. Structure of cannabidiol. VIII. Position of the double bonds in cannabidiol. Marihuana activity of tetrahydrocannabinols. *J Am Chem Soc* 1940;62:2566–2567.
51. Adams R, Baker BR. Structure of cannabidiol. VII.A. Method of synthesis of a tetrahydrocannabinol which possesses marihuana activity. *J Am Chem Soc* 1940;62:2405–2408.
52. Adams R, Pease DC, Cain CK, Baker BR, Clark JH, Wolff H, Wearn RB. Conversion of cannabidiol to a product with marihuana activity. A type reaction for synthesis of analogous substances. Conversion of cannabidiol to cannabinol. *J Am Chem Soc* 1940;62:2245–2246.
53. Ghosh R, Todd AR, Wilkinson S. *Cannabis indica*, Part IV. The synthesis of some tetrahydrodibenzopyran derivatives. *J Chem Soc* 1940; 1121–1125.
54. Krejčí Z, Šantavý F. Isolace dalších látek z listů indického konopí *Cannabis sativa L.* *Acta Univ Palacki Olomuc* 1955;6:59–66.
55. Kabelík J, Krejčí Z, Šantavý F. 1960; Cannabis as a medicament. *Bull Narc* 1960;12:5–23.
56. Mechoulam R, Shvo Y. The structure of cannabidiol. *Tetrahedron* 1963;19:2073–2078.
57. Gaoni Y, Mechoulam R. Isolation, structure, partial, synthesis of an active constituent of hashish. *J Am Chem Soc* 1964;86:1646–1647.
58. Šantavý F. Notes on the structure of cannabidiol compounds. *Acta Univ Palacki Olomuc, Fac Med* 1964;35:5–9.
59. Mechoulam R, Gaoni Y. The absolute configuration of Δ^1 -tetrahydrocannabinol, the major active constituent of hashish. *Tetrahedron Lett* 1967;8:1109–1111.
60. Hively RL, Mosher WA, Hoffmann FW. Isolation of *trans*- Δ^6 - tetrahydrocannabinol from marijuana. *J Am Chem Soc* 1966;88:1832–1833.
61. Gaoni Y, Mechoulam R. The structure and synthesis of cannabigerol, a new hashish constituent. *Proc Chem Soc, March* 1964; 82.
62. Gaoni Y, Mechoulam R. Cannabichromene, a new active principle in hashish. *Chem Comm* 1966; (1):20–21.
63. Claussen U, von Spulak F, Korte F. Zur chemischen klassifizierung von pflanzen XXXI. Haschisch X. Cannabichromen, ein neuer haschisch-inhalts-stoff. *Tetrahedron* 1966;22:1477–1479.
64. Crombie L, Ponsford R. Hashish components. Photochemical production of cannabicyclol from cannabichromene. *Tetrahedron Lett* 1968;9:5771–5772.
65. Mechoulam R, Gaoni Y. The isolation and structure of cannabinolic, cannabidiolic and cannabigerolic acids. *Tetrahedron* 1965;21:1223–1229.
66. Korte F, Haag M, Claussen U. Tetrahydrocannabinol-carbonsäure, ein neuer Haschisch-Inhaltsstoff. *Angew Chem* 1965;77:862.
67. Yamauchi T, Shoyama Y, Aramaki H, Azuma T, Nishioka I. Tetrahydrocannabinolic acid a genuine substance of tetrahydrocannabinol. *Chem Pharm Bull* 1967;15:1075–1076.
68. Mechoulam R, Ben-Zvi Z, Yagnitinsky B, Shani A. 1969; A new tetrahydrocannabinolic acid. *Tetrahedron Lett* 1969;10:2339–2341.
69. Hanuš L, Krejčí Z. Isolation of two new cannabinoid acids from *Cannabis sativa L.* of Czechoslovak origin. *Acta Univ Olomuc, Fac Med* 1975;74:161–166.
70. Shani A, Mechoulam R. Cannabielsoic acids. Isolation and synthesis by a novel oxidative cyclization. *Tetrahedron* 1974;30:2437–2446.
71. Gohda H, Narimatsu S, Watanabe K, Yamamoto I, Yoshimura H. The formation mechanism of cannabielsoin from cannabidiol with guinea-pig hepatic- microsomal enzymes. *J Pharm Sci* 1987;76:S32.
72. Mechoulam R, Ben-Zvi Z. Carboxylation of resorcinols with methyl magnesium carbonate. Synthesis of cannabinoid acids. *Chem Commun* 1969;(7):343–344.

73. Toyota M, Kinugawa T, Asakawa Y. Bibenzyl cannabinoid and bisbibenzyl derivative from the liverwort *Radula perrottetii*. *Phytochemistry* 1994;37:859–862.
74. Cullmann F, Becker H. Prenylated bibenzyls from the liverwort *Radula laxiramea*. *Z. Naturforsch. J Biosci* 1999;54:147–150.
75. Toyota M, Shimamura T, Ishii H, Renner M, Braggins J, Asakawa Y. New bibenzyl cannabinoid from the New Zealand liverwort *Radula marginata*. *Chem Pharm Bull* 2002;50:1390–1392.
76. Adams R. Isolation of cannabidiol from red oil obtained from hemp. US 2304669; 1942.
77. Todd AR. Hashish. *Experientia* 1946;2:55–60.
78. Schultz OE, Haffner G. The biosynthesis of cannabinol. III. *Arch Pharm* 1960;293:1–8.
79. Farmilo CG, McConnell DTW, Vandenheuvel FA, Lane R. Chemical analysis of marihuana. IV. Biogenesis, paper chromatography, gas chromatography. United Nations Document ST/SOA/SER. S/7, 1962.
80. Grlic L. A comparative study on some chemical and biological characteristics of various samples of cannabis resin. *Bull Narc* 1962;14:37–46.
81. Adams R, Pease DC, Cain CK, Clark JH. Structure of cannabinol. VI. Isomerization of cannabidiol to tetrahydrocannabinol, a physiologically active product. Conversion of cannabidiol to cannabinol. *J Am Chem Soc* 1940;62:2402–2405.
82. Simonsen JL, Todd AR. Cannabis indica. X. The essential oil from Egyptian hashish. *J Chem Soc* 1942; 188–191.
83. Todd AR. The hemp drugs. *Endeavour* 1943;2:69–72.
84. Farmilo CG. United Nations Document ST/SOA/SER. S/4, 1961.
85. Birch AJ. Biosynthetic relations of some natural phenolic and enolic compounds. *Fortschr Chem Org Naturstoffe* 1957;14:186–216.
86. Mechoulam R, Gaoni Y. Recent advances in the chemistry of hashish, Review article. *Fortschr Chem Org Naturstoffe* 1967;25:175–213.
87. Mechoulam R. Marihuana chemistry. Review. *Science* 1970;168:1159–1166.
88. Razdan RK. Recent advances in the chemistry of cannabinoids. *Progr Org Chem* 1973;8:78–101.
89. De Faubert Maunder MJ. A comparative evaluation of the tetrahydrocannabinol content of Cannabis plants. *J Assoc Pub Anal* 1970;8:42–47.
90. Shoyama Y, Yagi M, Nishioka I, Yamauchi T. Cannabis. 8. Biosynthesis of cannabinoid acids. *Phytochemistry* 1975;14:2189–2192.
91. Shoyama Y, Yamauchi T, Nishioka I. Cannabis. 5. Cannabigerolic acid monomethyl ether and cannabinolic acid. *Chem Pharm Bull* 1970;18:1327–1332.
92. Shoyama Y, Hirano H, Makino (née, Tomita) H, Umekita N, Nishioka I. Cannabis. 10. Isolation and structures of 4 new propyl cannabinoid acids, tetrahydrocannabivarinic acid, cannabidivarinic acid, cannabichromevarinic acid and cannabigerovarinic acid, from thai cannabis, Meao variant. *Chem Pharm Bull* 1977;25:2306–2311.
93. Shoyama Y, Nishioka I. Cannabis.13. 2 New spiro-compounds, cannabispinol and acetyl cannabispinol. *Chem Pharm Bull* 1978;26:3641–3646.
94. Crombie L, Crombie WML, Jamieson SV. Isolation of cannabispiradienone and cannabidihydrophenanthrene—Biosynthetic relationships between the spirans and dihydrostilbenes of Thailand cannabis. *Tetrahedron Lett* 1979;7:661–664.
95. Taura F, Morimoto S, Shoyama Y. Cannabis. 23. Cannabinerolic acid, a cannabinoid from *Cannabis sativa*. *Phytochemistry* 1995;39:457–458.
96. Taura F, Morimoto S, Shoyama Y, Mechoulam R. First direct evidence for the mechanism of Δ^1 -tetrahydrocannabinolic acid biosynthesis. *J Am Chem Soc* 1995;117:9766–9767.
97. Taura F, Shoyama Y, Morimoto S. Biosynthetic study on THCA, the psychoactive component of marijuana. *Seibutsu Butsuri* 2005;45:178–184.
98. Sirikantaramas S, Taura F, Tanaka Y, Ishikawa Y, Morimoto S, Shoyama Y. Tetrahydrocannabinolic acid synthase, the enzyme controlling marijuana psychoactivity, is secreted into the storage cavity of the glandular trichomes. *Plant Cell Physiol* 2005;46:1578–1582.
99. Sirikantaramas S, Morimoto S, Shoyama Y, Ishikawa Y, Wada Y, Shoyama Y, Taura F. The gene controlling marijuana psychoactivity: Molecular cloning and heterologous expression of Δ^1 -tetrahydrocannabinolic acid synthase from *Cannabis sativa L.* *J Biol Chem* 2004;279:39767–39774.
100. Shoyama Y, Takeuchi A, Taura F, Tamada T, Adachi M, Kuroki R, Shoyama Y, Morimoto S. Crystallization of Δ^1 -tetrahydrocannabinolic acid (THCA) synthase from *Cannabis sativa*. *Acta Crystallog* 2005;F61:799–801.
101. Turner CE, Hadley K. Constituents of *Cannabis sativa*. II. Absence of cannabidiol in an African variant. *J Pharm Sci* 1973;62:251–254.

102. Krejčí Z, Hanuš L, Yoshida T, Braenden OJ. The effect of climatic and ecologic conditions upon the formation and the amount of cannabinoid substances in the cannabis of various provenance. *Acta Univ Olomuc Fac Med* 1975;74:147–160.
103. GuerreroDavalos S, Fournier G, Boucher F, Paris M. Contribution to the study of Mexican marihuana. Preliminary studies: Cannabinoids and essential oil. *J Pharm Belg* 1977;32:89–99.
104. 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.
105. Morimoto S, Komatsu K, Taura F, Shoyama Y. Enzymological evidence for cannabichromenic acid biosynthesis. *J Nat Prod* 1997;60:854–857.
106. Morimoto S, Komatsu K, Taura F, Shoyama Y. Purification and characterization of cannabichromenic acid synthase from *Cannabis sativa*. *Phytochemistry* 1998;49:1525–1529.
107. Turner CE, El-Sohly MA. Constituents of *Cannabis sativa L.* XVI. Possible decomposition pathway of Δ^9 -tetrahydrocannabinol to cannabinol. *J Heterocycl Chem* 1979;16:1667–1668.
108. Takeya K, Itokawa H. Stereochemistry in oxidation of allylic alcohols by cell-free system of callus induced from *Cannabis sativa L.* *Chem Pharm Bull* 1977;25:1947–1951.
109. Itokawa H, Takeya K, Mihashi S. Biotransformation of cannabinoid precursors and related alcohols by suspension cultures of callus induced from *Cannabis sativa L.* *Chem Pharm Bull* 1977;25:1941–1946.
110. Heitrich A, Binder M. Identification of (3R,4R)-delta1(6)-tetrahydrocannabinol as an isolation artifact of cannabinoid acids formed by callus cultures of *Cannabis sativa L.* *Experientia* 1982;38:898–899.
111. Hartsel SC, Loh WHT, Robertson LW. Biotransformation of cannabidiol to cannabielsoin by suspension cultures of *Cannabis sativa* and *Saccharum officinarum*. *Planta med* 1983;48:17–19.
112. Loh WHT, Hartsel SC, Robertson LW. Tissue culture of *Cannabis sativa L.* and in vitro biotransformation of phenolics. *Z Pflanzenphysiol* 1983;111:395–400.
113. Turner CE, Mole ML, Hanuš L, El-Sohly MA. Constituents of *Cannabis sativa L.* Isolation of a new cannabinoid from an Indian variant of *Cannabis sativa L.* *Acta Univ Olomuc Fac Med* 1981;97:167–175.
114. Kajima M, Piraux M. The biogenesis of cannabinoids in *Cannabis sativa*. *Phytochemistry* 1982;21:67–69.
115. Shoyama Y, Hirano H, Nishioka I. Cannabis. Part 16. Biosynthesis of propyl cannabinoid acid and its biosynthetic relationship with pentyl and methyl cannabinoid acids. *Phytochemistry* 1984;23:1909–1912.
116. Hanuš L. Biogenesis of cannabinoid substances in the plant. *Acta Univ Palacki Olomuc, Fac Med* 1987;116:47–53.
117. Howlett AC. Inhibition of neuroblastoma adenylate cyclase by cannabinoid and nantradol compounds. *Life Sci* 1984;35:1803–1810.
118. Howlett AC, Champion TM, Wilken GH, Mechoulam R. Stereochemical effects of 11-OH- Δ^8 -tetrahydrocannabinol-dimethylheptyl to inhibit adenylate cyclase and bind to the cannabinoid receptor. *Neuropharmacology* 1990;29:161–165.
119. Howlett AC, Fleming RM. Cannabinoid inhibition of adenylate cyclase. Pharmacology of the response in neuroblastoma cell membranes. *Mol Pharmacol* 1984;26:532–538.
120. Howlett AC, Qualy JM, Khachatrian LL. Involvement of Gi in the inhibition of adenylate cyclase by cannabimimetic drugs. *Mol Pharmacol* 1986;29:307–313.
121. Devane WA, Dysarz FA III, Johnson MR, Melvin LS, Howlett AC. Determination and characterization of a cannabinoid receptor in rat brain. *Mol Pharmacol* 1988;34:605–613.
122. Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature* 1990;346:561–564.
123. Gérard CM, Mollereau C, Vassart G, Parmentier M. Molecular cloning of a human cannabinoid receptor which is also expressed in testis. *Biochem J* 1991;279:129–134.
124. Gérard C, Mollereau C, Vassart G, Parmentier M. Nucleotide sequence of a human cannabinoid receptor cDNA. *Nucleic Acids Res* 1990;18:7142.
125. Chakrabarti A, Onaivi ES, Chaudhuri G. Cloning and sequencing of a cDNA encoding the mouse brain-type cannabinoid receptor protein. *DNA Seq* 1995;5:385–3388.
126. Munro S, Thomas KL, Abu-Shaar M. Molecular characterization of a peripheral receptor for cannabinoids. *Nature* 1993;365:61–65.
127. Shire D, Calandra B, Rinaldi-Carmona M, Oustric D, Pessegue B, Bonnin-Cabanne O, Le Fur G, Caput D, Ferrara P. Molecular cloning, expression and function of the murine CB₂ peripheral cannabinoid receptor. *Biochim Biophys Acta* 1996;1307:132–136.
128. Griffin G, Tao Q, Abood ME. Cloning and pharmacological characterization of the rat CB(2) cannabinoid receptor. *J Pharmacol Exp Ther* 2000;292:886–894.

129. Van Sickle MD, Duncan M, Kingsley PJ, Mouihate A, Urbani P, Mackie K, Stella N, Makriyannis A, Piomelli D, Davison JS, Marnett LJ, Di Marzo V, Pittman QJ, Patel KD, Sharkey KA. Identification and functional characterization of brainstem cannabinoid CB₂ receptors. *Science* 2005;310:329–332.
130. Howlett AC, Barth F, Bonner TI, Cabral G, Casellas P, Devane WA, Felder CC, Herkenham M, Mackie K, Martin BR, Mechoulam R, Pertwee RG. International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. *Pharmacol Rev* 2002;54:161–202.
131. Gong J-P, Onaivi ES, Ishiguro H, Liu Q-R, Tagliaferro PA, Brusco A, Uhl GR. Cannabinoid CB₂ receptors: Immunohistochemical localization in rat brain. *Brain Res* 2006;1071:10–23.
132. Onaivi ES, Ishiguro H, Gong J-P, Patel S, Perchuk A, Meozzi PA, Myers L, Mora Z, Tagliaferro P, Gardner E, Brusco A, Akinshola BE, Liu Q-R, Hope B, Iwasaki S, Arinami T, Teasentz L, Uhl GR. Discovery of the presence and functional expression of cannabinoid CB₂ receptors in brain. *Ann NY Acad Sci* 2006;1074:514–536.
133. Wagner JA, Varga K, Jarai Z, Kunos G. Mesenteric vasodilation mediated by endothelial anandamide receptors. *Hypertension* 1999;33:429–434.
134. Sawzdargo M, Nguyen T, Lee DK, Lynch KR, Cheng R, Heng HHQ, George SR, O'Dowd BF. Identification and cloning of three novel human G protein-coupled receptor genes GPR52, Ψ GPR53 and GPR55: GPR55 is extensively expressed in human brain. *Mol Brain Res* 1999;64:193–198.
135. Drmota T, Greasley P, Groblewski T. Screening assays for cannabinoid ligand- GPR55 receptor binding modulators. WO2004/074844, 2004; 49 p.
136. Ryberg E, Larsson N, Sjoegren S, Hjorth S, Hermansson N-O, Leonova J, Elebring T, Nilsson K, Drmota T, Geasley PJ. The orphan receptor GPR55 is a novel cannabinoid receptor. *Br J Pharmacol* 2007;152:1092–1101.
137. Petitet F, Donlan M, Michel A. GPR55 as a new cannabinoid receptor: Still a long way to prove it. *Chem Biol Drug Design* 2006;67:252–253.
138. Oka S, Nakajima K, Yamashita A, Kishimoto S, Sugiura T. Identification of GPR55 as a lysophosphatidylinositol receptor. *Biochem Biophys Res Commun* 2007;362:928–934.
139. Milman G, Maor Y, Abu-Lafi S, Horowitz M, Gallily R, Batkai S, Mo FM, Offertaler L, Pacher P, Kunos G, Mechoulam R. N-Arachidonoyl L-serine, an endocannabinoid-like brain constituent with vasodilatory properties. *Proc Natl Acad Sci* 2006;103:2428–2433.
140. Offertaler L, Mo FM, Batkai S, Liu J, Begg M, Razdan RK, Martin BR, Bukoski RD, Kunos G. Selective ligands and cellular effectors of a G protein-coupled endothelial cannabinoid receptor. *Mol Pharmacol* 2003;63:699–705.
141. Begg M, Pacher P, Batkai S, Osei-Hyiaman D, Offertaler L, Mo FM, Liu H, Kunos G. Evidence for novel cannabinoid receptors. *Pharmacol Therapeut* 2005;106:133–145.
142. Pertwee RG. GPR55: A new member of the cannabinoid receptor clan? *Br J Pharmacol* 2007;152:984–986.
143. Devane WA, Hanuš L, Breuer A, Pertwee RG, Stevenson LA, 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.
144. Hanuš LO. Discovery and isolation of anandamide and other endocannabinoids. *Chem Biodiv* 2007;4:1828–1841.
145. Hanuš L, Gopher A, Almog S, Mechoulam R. Two new unsaturated fatty acid ethanolamides in brain that bind to the cannabinoid receptor. *J Med Chem* 1993;36:3032–3034.
146. Porter AC, Sauer JM, Knierman MD, Becker GW, Berna MJ, Bao JQ, Nomikos GG, Carter P, Bymaster FP, Leese AB, Felder CC. Characterization of a novel endocannabinoid, virodhamine, with antagonist activity at the CB₁ receptor. *J Pharmacol Exp Ther* 2002;301:1020–1024.
147. Mechoulam R, Ben-Shabat S, Hanuš L, Ligumsky M, Kaminski NE, Schatz AR, Gopher A, Almog S, Martin BR, Compton DR, Pertwee RG, 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.
148. Sugiura T, Kondo S, Sukagawa A, Nakane S, Shinoda A, Itoh K, Yamashita A, Waku K. 2-Arachidonoylglycerol: A possible endogenous cannabinoid receptor ligand in brain. *Biochem Biophys Res Commun* 1995;215:89–97.
149. Hanuš L, Abu-Lafi S, Fride E, Breuer A, Vogel Z, Shalev DE, Kustanovich I, Mechoulam R. 2-arachidonoyl glyceryl ether, an endogenous agonist of the cannabinoid CB₁ receptor. *Proc Natl Acad Sci* 2001;98:3662–3665.
150. Huang SM, Bisogno T, Trevisani M, Al-Hayani A, De Petrocellis L, Fezza F, Tognetto M, Petros TJ, Krey JF, Chu CJ, Miller JD, Davies SN, Geppetti P, Walker JM, Di Marzo V. An endogenous capsaicin-like

- substance with high potency at recombinant and native vanilloid VR1 receptors. *Proc Natl Acad Sci* 2002;99:8400–8405.
151. Ross RA. Anandamide and vanilloid TRPV1 receptors. *Br J Pharmacol* 2003;140:790–801.
 152. De Petrocellis L, Chu CJ, Moriello AS, Kellner JC, Walker JM, Di Marzo V. Actions of two naturally occurring saturated N-acyldopamines on transient receptor potential vanilloid 1 (TRPV1) channels. *Br J Pharmacol* 2004;143:251–256.
 153. Bisogno T, Melck D, Bobrov MY, Gretskaya NM, Bezuglov VV, De Petrocellis L, Di Marzo V. N-acyldopamines: Novel synthetic CB(1) cannabinoid-receptor ligands and inhibitors of anandamide inactivation with cannabimimetic activity in vitro and in vivo. *Biochem J* 2000;351:817–824.
 154. Mechoulam R, Frideri E, Di Marzo V. Endocannabinoids. *Eur J Pharmacol* 1998;359:1–18.
 155. Di Marzo V, De Petrocellis L, Bisogno T, Melck D. Metabolism of anandamide and 2-arachidonoylglycerol: An historical overview and some recent developments. *Lipids* 1999;34:S319–S325.
 156. Giuffrida A, Beltramo M, Piomelli D. Mechanisms of endocannabinoid inactivation: Biochemistry and pharmacology. *J Pharmacol Exp Ther* 2001;298:7–14.
 157. Hillard CJ. Biochemistry and pharmacology of the endocannabinoids arachidonylethanolamide and 2-arachidonoylglycerol. *Prostagl Other Lipid Mediat* 2000;61:3–18.
 158. Schmid HH. Pathways and mechanisms of N-acylethanolamine biosynthesis: Can anandamide be generated selectively? *Chem Phys Lipids* 2000;108:71–87.
 159. Sugiura T, Kobayashi Y, Oka S, Waku K. Biosynthesis and degradation of anandamide and 2-arachidonoylglycerol and their possible physiological significance. *Prostagl Leukot Ess Fatty Acids* 2002;66:173–192.
 160. Okamoto Y, Wang J, Morishita J, Ueda N. Biosynthetic pathways of the endocannabinoid anandamide. *Chem Biodiv* 2007;4:1842–1857.
 161. Natarajan V, Schmid PC, Reddy PV, Schmid HHO. Catabolism of N-acylethanolamine phospholipids by dog brain preparations. *J Neurochem* 1984;42:1613–1619.
 162. Simon GM, Cravatt BF. Endocannabinoid biosynthesis proceeding through glycerophospho-N-acylethanolamine and a role for α/β -hydrolase 4 in this pathway. *J Biol Chem* 2006;281:26465–26472.
 163. Liu J, Wang L, Harvey-White J, Osei-Hyiaman D, Razdan R, Gong Q, Chan AC, Zhou Z, Huang BX, Kim H-Y, Kunos G. A biosynthetic pathway for anandamide. *Proc Natl Acad Sci* 2006;103:13345–13350.
 164. Liu J, Wang L, Harvey-White J, Huang BX, Kim H-Y, Luquet S, Palmiter RD, Krystal G, Rai R, Mahadevan A, Razdan RK, Kunos G. Multiple pathways involved in the biosynthesis of anandamide. *Neuropharmacology* 2008;54:1–7.
 165. Schmid HH, Schmid PC, Natarajan V. N-acylated glycerophospholipids and their derivatives. *Prog Lip Res* 1990;29:1–43.
 166. Schmid HHO. Pathways and mechanisms of N-acylethanolamine biosynthesis: Can anandamide be generated selectively? *Chem Phys Lip* 2000;108:71–87.
 167. Cadas H, di Tomaso E, Piomelli D. Occurrence and biosynthesis of endogenous cannabinoid precursor, N-arachidonoyl phosphatidylethanolamine, in rat brain. *J Neurosci* 1997;17:1226–1242.
 168. Hansen HS, Moesgaard B, Petersen G, Hansen HH. Putative neuroprotective actions of N-acylethanolamines. *Pharmacol Ther* 2002;95:119–126.
 169. Jin X-H, Okamoto Y, Morishita J, Tsuboi K, Tonai T, Ueda N. Discovery and characterization of a Ca^{2+} -independent phosphatidylethanolamine N-acyltransferase generating the anandamide precursor and its congeners. *J Biol Chem* 2007;282:3614–3623.
 170. Astarita G, Ahmed F, Piomelli D. Identification of biosynthetic precursors for the endocannabinoid anandamide in the rat brain. *J Lip Res* 2008;49:48–57.
 171. Snider NT, Kornilov AM, Kent UM, Hollenberg PF. Anandamide metabolism by human liver and kidney microsomal cytochrome P450 enzymes to form hydroxyeicosatetraenoic and epoxyeicosatrienoic acid ethanolamides. *J Pharmacol Exp Ther* 2007;321:590–597.
 172. van der Stelt M, van Kuik JA, Bari M, van Zadelhoff G, Leeftang BR, Veldink GA, Finazzi-Agro A, Vliegthart JFG, Maccarrone M. Oxygenated metabolites of anandamide and 2-arachidonoylglycerol: Conformational analysis and interaction with cannabinoid receptors, membrane transporter, and fatty acid amide hydrolase. *J Med Chem* 2002;45:3709–3720.
 173. Kozak KR, Rowlinson SW, Marnett LJ. Oxygenation of the endocannabinoid, 2-arachidonoylglycerol, to glyceryl prostaglandins by cyclooxygenase-2. *J Biol Chem* 2000;275:33744–33749.
 174. Kozak KR, Marnett LJ. Oxidative metabolism of endocannabinoids. *Prostagl Leukotr Ess Fatty Acids* 2002;66:211–220.
 175. Kozak KR, Crews BC, Morrow JD, Wang L-H, Ma YH, Weinander R, Jakobsson P-J, Marnett LJ. Metabolism of the endocannabinoids, 2-arachidonoylglycerol and anandamide, into prostaglandin, thromboxane, and prostacyclin glycerol esters and ethanolamides. *J Biol Chem* 2002;277:44877–44885.

176. Kozak KR, Prusakiewicz JJ, Marnett LJ. Oxidative metabolism of endocannabinoids by COX-2. *Curr Pharm Design* 2004;10:659–667.
177. Yang W, Ni J, Woodward DF, Tang-Liu DD-S, Ling K-HJ. Enzymatic formation of prostamide F_{2α} from anandamide involves a newly identified intermediate metabolite, prostamide H₂. *J Lip Res* 2005;46:2745–2751.
178. Dickason-Chesterfield AK, Kidd SR, Moore SA, Schaus JM, Liu B, Nomikos GG, Felder CC. Pharmacological characterization of endocannabinoid transport and fatty acid amide hydrolase inhibitors. *Cell Mol Neurobiol* 2006;26:405–421.
179. Moore SA, Nomikos GG, Dickason-Chesterfield AK, Schober DA, Schaus JM, Ying BP, Xu YC, Phebus L, Simmons RMA, Li D, Lyengar S, Felder CC. Identification of a high-affinity binding site involved in the transport of endocannabinoids. *Proc Natl Acad Sci* 2005;102:17852–17857.
180. Beltramo M, Stella N, Calignano A, Lin SY, Makriyannis A, Piomelli D. Functional role of high-affinity anandamide transport, as revealed by selective inhibition. *Science* 1997;277:1094–1097.
181. Beltramo M, Stella N, Calignano A, Lin SY, Makriyannis A, Piomelli D. Functional role of high-affinity anandamide transport, as revealed by selective inhibition. *Science* 1997;277:1094–1097.
182. Bisogno T, Hanuš L, De Petrocellis L, Tchilibon S, Ponde DE, Brandi I, Moriello AS, Davis JB, 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;135:845–852.
183. De Petrocellis L, Bisogno T, Davis JB, Pertwee RG, Di Marzo V. Overlap between the ligand recognition properties of the anandamide transporter and the VR1 vanilloid receptor: Inhibitors of anandamide uptake with negligible capsaicin-like activity. *FEBS Lett* 2000;483:52–56.
184. Vandevoorde S, Fowler CJ. Inhibition of fatty acid amide hydrolase and monoacylglycerol lipase by the anandamide uptake inhibitor VDM11: Evidence that VDM11 acts as an FAAH substrate. *Br J Pharmacol* 2005;145:885–893.
185. Lopez-Rodriguez ML, Viso A, Ortega-Gutierrez S, Fowler CJ, Tiger G, de Lago E, Fernandez-Ruiz J, Ramos JA. Design, synthesis and biological evaluation of new endocannabinoid transporter inhibitors. *Eur J Med Chem* 2003;38:403–412.
186. de Lago E, Fernandez-Ruiz J, Ortega-Gutierrez S, Viso A, Lopez-Rodriguez ML, Ramos JA. UCM707, a potent and selective inhibitor of endocannabinoid uptake, potentiates hypokinetic and antinociceptive effects of anandamide. *Eur J Pharmacol* 2002;449:99–103.
187. Fowler JJ, Jacobsson SOP. Cellular transport of anandamide, 2-arachidonoylglycerol and palmitoylethanolamide—Targets for drug development? *Prostagl Leukotr Ess Fatty Acids* 2002;66:193–200.
188. Deutsch DG, Ueda N, Yamamoto S. The fatty acid amide hydrolase (FAAH). *Prostagl Leukotr Ess Fatty Acids* 2002;66:201–210.
189. Fowler CJ, Jonsson KO, Tiger G. Fatty acid amide hydrolase: Biochemistry, pharmacology, and therapeutic possibilities for an enzyme hydrolyzing anandamide, 2-arachidonoylglycerol, palmitoylethanolamide, and oleamide. *Biochem Pharmacol* 2001;62:517–526.
190. Boger DL, Henriksen SJ, Cravatt BF. Oleamide: An endogenous sleep-inducing lipid and prototypical member of a new class of biological signaling molecules. *Curr Pharm Design* 1998;4:303–314.
191. Deutsch DG, Omeir R, Arreaza G, Salehani D, Prestwich GD, Huang Z, Howlett A. Methyl arachidonyl fluorophosphonate: A potent irreversible inhibitor of anandamide amidase. *Biochem Pharmacol* 1997;53:255–260.
192. Martin BR, Beletskaya I, Patrick G, Jefferson R, Winckler R, Deutsch DG, Di Marzo V, Dasse O, Mahadevan A, Razdan RK. Cannabinoid properties of methylfluorophosphonate analogs. *J Pharmacol Exp Ther* 2000;294:1209–1218.
193. Fernando SR, Perwee RG. Evidence that methyl arachidonyl fluorophosphonate is an irreversible cannabinoid receptor antagonist. *Br J Pharmacol* 1997;121:1716–1720.
194. Muccioli GG, Xu C, Odah E, Cudaback E, Cisneros JA, Lambert DM, Rodriguez MLL, Bajjalieh S, Stella N. Identification of a novel endocannabinoid—Hydrolyzing enzyme expressed by microglial cells. *J Neurosci* 2007;27:2883–2889.
195. Bachur NR, Mašek K, Melmon KL. Fatty acid amides of ethanolamine in mammalian tissues. *J Biol Chem* 1965;240:1019–1024.
196. Conti S, Costa B, Colleoni M, Parolaro D, Giagnoni G. Antiinflammatory action of endocannabinoid palmitoylethanolamide and the synthetic cannabinoid nabilone in a model of acute inflammation in the rat. *Br J Pharmacol* 2002;135:181–187.
197. Lambert DM, Vandevoorde S, Jonsson KO, Fowler CJ. 2002; The palmitoylethanolamide family: A new class of anti-inflammatory agents? *Curr Med Chem* 2002;9:663–674.

198. Ben-Shabat S, Fride E, Sheskin T, Tamiri T, Rhee M-H, Vogel Z, Bisogno T, De Petrocellis L, Di Marzo V, Mechoulam R. 1998; An entourage effect: Inactive endogenous fatty acid glycerol esters enhance 2-arachidonoyl-glycerol cannabinoid activity. *Eur J Pharmacol* 1998;353:23–31.
199. Terrazzino S, Berto F, Carbonare MD, Fabris M, Guiotto A, Bernardini D, Leon A. Stearoylethanolamide exerts anorexic effects in mice via downregulation of liver stearyl-coenzyme A desaturase-1 mRNA expression. *FASEB J* 2004;18:1580–1582.
200. Maccarrone M, Cartoni A, Parolaro D, Margonelli A, Massi P, Bari M, Battista N, Finazzi-Agro A. Cannabimimetic activity, binding, and degradation of stearoylethanolamide within the mouse central nervous system. *Mol Cell Neurosci* 2002;21:126–140.
201. Maccarrone M, Pauselli R, di Rienzo M, Finazzi-agro A. Binding, degradation and apoptotic activity of stearoylethanolamide in rat C6 glioma cells. *Biochem J* 2002;366:137–144.
202. Rodriguez de Fonseca F, Navarro M, Gómez R, Escuredo L, Nava F, Fu J, Murillo-Rodríguez E, Giuffrida A, LoVerme J, Gaetani S, Kathuria S, Gall C, Piomelli D. An anorexic lipid mediator regulated by feeding. *Nature* 2001;414:209–212.
203. Brito B, Castro R, Cabrera de Leon A. Oleoylethanolamide: A molecular cross-talk with leptin in feeding behaviour regulation. *Lett Drug Des Disc* 2006;3:741–746.
204. Ambrosini A, Zolse G, Ambrosi S, Ragni L, Tiano L, Littarru G, Bertoli E, Mantero F, Boscaro M, Balercia G. Oleoylethanolamide protects humans sperm cells from oxidation stress: Studies on cases of idiopathic infertility. *Biol Reprod* 2006;74:659–665.
205. Cravatt BF, Prospero-Garcia O, Siuzdak G, Gilula NB, Henriksen SJ, Boger DL, Lerner RA. Chemical characterization of a family of brain lipids that induce sleep. *Science* 1995;268:1506–1509.
206. Hanuš LO, Fales HM, Spande TF, Basile AS. A gas chromatographic-mass spectral assay for the quantitative determination of oleamide in biological fluids. *Anal Biochem* 1999;270:159–166.
207. Basile AS, Hanuš L, Mendelson WB. Characterization of the hypnotic properties of oleamide. *Neuro Report* 1999;10:947–951.
208. Leggett JD, Aspley S, Beckett SRG, D'Antona AM, Kendall DA, Kendall DA. Oleamide is a selective endogenous agonist of rat and human CB₁ cannabinoid receptors. *Br J Pharmacol* 2004;141:253–262.
209. Hoi PM, Hiley CR. Vasorelaxant effects of oleamide in rat small mesenteric artery indicate action at a novel cannabinoid receptor. *Br J Pharmacol* 2006;147:560–568.
210. Lambert DM, Di Marzo V. The palmitoylethanolamide and oleamide enigmas: Are these two fatty acid amides cannabimimetic? *Curr Med Chem* 1999;6:757–773.
211. Saghatelian A, Trauger SA, Want EJ, Hawkins EG, Siuzdak G, Cravatt BF. Assignment of endogenous substrates to enzymes by global metabolite profiling. *Biochemistry* 2004;43:14332–14339.
212. Saghatelian A, McKinney MK, Bandell M, Patapoutian A, Cravatt BF. A FAAH-regulated class of N-acyl taurines that activates TRP ion channels. *Biochemistry* 2006;45:9007–9015.
213. Johnson MR, Melvin LS. 2-Hydroxy-4-(substituted) phenyl cycloalkanes and derivatives. United States Patent 4,371,720; February 1, Pfizer Inc., New York, NY; 1983; 46 pp.
214. Melvin LS, Johnson MR, Milne GM. A cannabinoid derived analgesic (CP-55,940). In *Abstracts of Papers, 186th Natl. Meet. American Chemical. Soc., Washington, D.C., August 1983, American Chemical Society, Washington, D.C.; Abstr. MEDI, 2.*
215. Burstein SH, Rossetti RG, Yagen B, Zurier RB. Oxidative metabolism of anandamide. *Prostag Oth Lipid M* 2000;61:29–41.
216. Johnson MR, Melvin LS. The discovery of nonclassical cannabinoid analgetics. In: Mechoulam R, editor. *Cannabinoids as therapeutic agents Chapter 7.* Boca Raton, FL: CRC Press Inc.; 1986. pp. 121–145.
217. Gerard CM, Mollereau C, Vassart G, Parmentier M. Molecular-cloning of a human cannabinoid receptor which is also expressed in testis. *Biochem J* 1991;279:129–134.
218. Mechoulam R, Feigenbaum JJ, Lander N, Segal M, Jarbe TUC, Hiltunen AJ, Consroe P. Enantiomeric cannabinoids: Stereospecificity of psychotropic activity. *Experientia* 1988;44:762–764.
219. Mechoulam R, Lander N, Breuer A, Zahalka J. Synthesis of the individual, pharmacologically distinct, enantiomers of a tetrahydrocannabinol derivative. *Tetrahedron Asymmetr* 1990;1:315–319.
220. Little PJ, Compton DR, Mechoulam R, Martin B. Stereochemical effects of 11-OH-delta-8-THC-dimethylheptyl in mice and dogs. *Pharmacol Biochem Behav* 1989;32:661–666.
221. Jarbe TUC, Hiltunen AJ, Mechoulam R. Stereospecificity of the discriminative stimulus functions of the dimethylheptyl homologs of 11-OH-delta-8-tetrahydrocannabinol in rats and pigeons. *J Pharmacol Exper Ther* 1989;250:1000–1005.
222. Devane WA, Breuer A, Sheskin T, Jarbe TUC, Eisen M, Mechoulam R. A novel probe for the cannabinoid receptor. *J Med Chem* 1992;35:2065–2069.
223. Vann RE, Cook CD, Martin BR, Wiley JL. Cannabimimetic properties of ajulemic acid. *J Pharmacol Exp Ther* 2007;320:678–686.

224. Burstein SH, Audette CA, Breuer A, Devane WA, Colodner S, Doyle A, Mechoulam R. Synthetic nonpsychotropic cannabinoids with potent antiinflammatory, analgesic, and leukocyte antiadhesion activities. *J Med Chem* 1992;35:3135–3141.
225. Burstein SH. Ajulemic acid (CT3): A potent analog of the acid metabolites of THC. *Curr Pharm Design* 2000;6:1339–1345.
226. Burstein SH, Karst M, Schneider U, Zurier RB. Ajulemic acid: A novel cannabinoid produces analgesia without a “high”. *Life Sci* 2004;75:1513–1522.
227. Recht LD, Salmons R, Rosetti R, Jang T, Pipia G, Kubiowski T, Karim P, Ross AH, Zurier R, Litofsky NS, Burstein S. Antitumor effects of ajulemic acid (CT3), a synthetic non-psychoactive cannabinoid. *Biochem Pharmacol* 2001;62:755–763.
228. Liu J-L, Li H, Burstein SH, Zurier RB, Chen JD. Activation and binding of peroxisome proliferator-activated receptor γ by synthetic cannabinoid ajulemic acid. *Mol Pharmacol* 2003;63:983–992.
229. Bidinger B, Torres R, Rossetti RG, Brown L, Beltre R, Burstein S, Lian JB, Stein GS, Zuriera RB. Ajulemic acid, a nonpsychoactive cannabinoid acid, induces apoptosis in human T lymphocytes. *Clin Immunol* 2003;108:95–102.
230. Hanuš L, Breuer A, Tchilibon S, Shiloah S, Goldenberg D, Horowitz M, Fride E, Mechoulam R. HU-308: A specific agonist for CB₂, a peripheral cannabinoid receptor. *Proc Natl Acad Sci* 1999;96:14228–14233.
231. Pharms Corp: Bicyclic cannabinoid. Poster, Society for Neuroscience - 32nd Annual Meeting, 3-7 November 2002, Orlando, FL, USA.
232. Huffman JW, Yu S, Showalter V, Abood ME, Wiley JL, Compton DR, Martin BR, Bramblett RD, Reggio PH. Synthesis and pharmacology of a very potent cannabinoid lacking a phenolic hydroxyl with high affinity for the CB₂ receptor. *J Med Chem* 1996;39:3875–3877.
233. Huffman JW, Liddle J, Yu S, Aung MM, Abood ME, Wiley JL, Martin BR. 3-(1',1'-Dimethylbutyl)-1-deoxy- Δ^8 -THC and related compounds: Synthesis of selective ligands for the CB₂ receptor. *Bioorg Med Chem* 1999;7:2905–2914.
234. Huffman JW, Bushell SM, Miller JRA, Wiley JL, Martin BR. 1-methoxy-, 1-deoxy-11-hydroxy- and 11-hydroxy-1-methoxy- Δ^8 -tetrahydrocannabinols: New selective ligands for the CB₂ receptor. *Bioorg Med Chem* 2002;10:4119–4129.
235. Makriyannis A, Lai XZ, Lu D. Preparation of novel biphenyl and biphenyl-like cannabinoids with binding affinities for the CB₁ and CB₂ cannabinoid receptor. Univ. Connecticut: WO04017920 (2004).
236. Razdan RK, Martin BR. Cannabinoids. Virginia Commonwealth Univ.: WO03091189 (2003).
237. Ross RA, Brockie HC, Stevenson LA, Murphy VL, Templeton F, Makriyannis A, Pertwee RG. Agonist-inverse agonist characterization at CB₁ and CB₂ cannabinoid receptors of L759633, L759656 and AM630. *Br J Pharmacol* 1999;126:665–672.
238. Huffman JW, Bushell SM, Joshi SN, Wiley JL, Martin BR. Enantioselective synthesis of 1-methoxy- and 1-deoxy-2'-methyl- Δ^8 -tetrahydrocannabinols: New selective ligands for the CB₂ receptor. *Bioorg Med Chem* 2006;14:247–262.
239. Marriott K-SC, Huffman JW, Wiley JL, Martin BR. Synthesis and pharmacology of 11-nor-1-methoxy-9-hydroxyhexahydrocannabinols and 11-nor-1-deoxy-9-hydroxyhexahydrocannabinols: New selective ligands for the cannabinoid CB₂ receptor. *Bioorg Med Chem* 2006;14:2386–2397.
240. Durdagi S, Kapou A, Kourouli T, Andreou T, Nikas SP, Nahmias VR, Papahatjis DP, Papadopoulos MG, Mavromoustakos T. The application of 3D-QSAR studies for novel cannabinoid ligands substituted at the C1' position of the alkyl side chain on the structural requirements for binding to cannabinoid receptors CB₁ and CB₂. *J Med Chem* 2007;50:2875–2885.
241. Martin B, Stevenson LA, Pertwee RG, Breivogel CS, Williams W, Mahadevan A, Razdan RK. Agonists and silent antagonists in a series of cannabinoid sulfonamides. Symposium on the Cannabinoids. Burlington, Vermont: International Cannabinoid Research Society; 2002. p. 2.
242. Martin BR, Razdan RK, Pertwee RG. Preparation of tetrahydrocannabinolsulfonamides as a silent agonist of the CB₁ cannabinoid receptor. US Patent 2005096379; 2005; 16 pp.
243. Gardner A, Mallet PE. Suppression of feeding, drinking, and locomotion by a putative cannabinoid receptor 'silent antagonist'. *Eur J Pharmacol* 2006;530:103–106.
244. Thomas A, Ross RA, Saha B, Mahadevan A, Razdan RK, Pertwee RG. 6''-Azidohex-2''-yne-cannabidiol: A potential neutral, competitive cannabinoid CB₁ receptor antagonist. *Eur J Pharmacol* 2004;487:213–221.
245. Sumariwalla PF, Gallily R, Tchilibon S, Fride E, Mechoulam R, Feldmann M. A novel synthetic, nonpsychoactive cannabinoid acid (HU-320) with antiinflammatory properties in murine collagen-induced arthritis. *Arthritis Rheum* 2004;50:985–998.

246. Abadji V, Lin S, Taha G, Griffin G, Stevenson LA, Pertwee RG, Makriyannis A. (R)-methanandamide: A chiral novel anandamide possessing higher potency and metabolic stability. *J Med Chem* 1994;37:1889–1893.
247. Khanolkar AD, Abadji V, Lin S, Hill WAG, Taha G, Abouzid K, Meng Z, Fan P, Makriyannis A. Head group analogs of arachidonylethanolamide, the endogenous cannabinoid ligand. *J Med Chem* 1996; 39:4515–4519.
248. Sheskin T, Hanuš L, Slager Y, Vogel Z, Mechoulam R. Structural requirements for binding of anandamide-type compounds to the brain cannabinoid receptor. *J Med Chem* 1997;40:659–667.
249. Parkkari T, Savinainen JR, Raitio KH, Saario SM, Matilainen L, Sirvioe T, Laitinen JT, Nevalainen T, Niemi R, Jaervinen T. Synthesis, cannabinoid receptor activity, and enzymatic stability of reversed amide derivatives of arachidonoyl ethanolamide. *Bioorg Med Chem* 2006;14:5252–5258.
250. Parkkari T, Salo OMH, Huttunen KM, Savinainen JR, Laitinen JT, Poso A, Nevalainen T, Jaervinen T. Synthesis and CB₁ receptor activities of dimethylheptyl derivatives of 2-arachidonoyl glycerol (2-AG) and 2-arachidonoyl glyceryl ether (2-AGE). *Bioorg Med Chem* 2006;14:2850–2858.
251. Parkkari T, Myllymaeki M, Savinainen JR, Saario SM, Castillo-Melendez JA, Laitinen JT, Nevalainen T, Koskinen AMP, Jaervinen T. α -Methylated derivatives of 2-arachidonoyl glycerol: Synthesis, CB₁ receptor activity, and enzymatic stability. *Bioorg Med Chem Lett* 2006;16:2437–2440.
252. Bisogno T, Cascio MG, Saha B, Mahadevan A, Urbani P, Minassi A, Appendino G, Saturnino C, Martin B, Razdan R, Di Marzo V. Development of the first potent and specific inhibitors of endocannabinoid biosynthesis. *Biochim Biophys Acta Mol Cell Biol Lip* 2006;1761:205–212.
253. Raduner S, Majewska A, Chen J-Z, Xie X-Q, Hamon J, Faller B, Altmann K-H, Gertsch J. Alkylamides from echinacea are a new class of cannabinomimetics: Cannabinoid type 2 receptor-dependent and -independent immunomodulatory effects. *J Biol Chem* 2006;28:14192–14206.
254. Urbani P, Cavallo P, Cascio MG, Buonerba M, De Martino G, Di Marzo V, Saturnino C. New metabolically stable fatty acid amide ligands of cannabinoid receptors: Synthesis and receptor affinity studies. *Bioorg Med Chem Lett* 2006;16:138–141.
255. Ligresti A, Cascio MG, Pryce G, Kulasegram S, Beletskaya I, De Petrocellis L, Saha B, Mahadevan A, Visintin C, Wiley JL, Baker D, Martin BR, Razdan RK, Di Marzo V. New potent and selective inhibitors of anandamide reuptake with antispastic activity in a mouse model of multiple sclerosis. *Br J Pharmacol* 2006;147:83–91.
256. Appendino G, Minassi A, Berton L, Moriello AS, Cascio MG, De Petrocellis L, Di Marzo V. Oxyhomologues of anandamide and related endolipids: Chemoselective synthesis and biological activity. *J Med Chem* 2006;49:2333–2338.
257. Bourne C, Roy S, Wiley JL, Martin BR, Thomas BF, Mahadevan A, Razdan RK. Novel, potent THC/anandamide (hybrid) analogs. *Bioorg Med Chem* 2007;15:7850–7864.
258. Bell MR, D'Ambra TE, Kumar V, Eissenstat MA, Herrmann JL Jr, Wetzel JR, Rosi D, Pillion RE, Daum SJ, Hlasta DJ, Kullnig RK, Ackerman JH, Haubrich DR, Luttinger DA, Baizman ER, Miller MS, Ward SJ. Antinociceptive (aminoalkyl)indoles. *J Med Chem* 1991;34:1099–1110.
259. D'Ambra TE, Estep KG, Bell MR, Eissenstat MA, Josef KA, Ward SJ, Haycock DA, Baizman ER, Casiano FM, Beglin NC, Chippari SM, Grego JD, Kullnig RK, Daley GT. Conformationally restrained analogs of pravadoline: Nanomolar potent, enantioselective, (aminoalkyl)indole agonists of the cannabinoid receptor. *J Med Chem* 1992;35:124–135.
260. Eissenstat MA, Bell MR, D'Ambra TE, Alexander EJ, Daum SJ, Ackerman JH, Gruett MD, Kumar V, Estep KG, Olefirowicz EM, Wetzel JR, Alexander MD, Weaver JD III, Haycock DA, Luttinger DA, Casiano FM, Chippari SM, Kuster JE, Stevenson JI, Ward SJ. Aminoalkylindoles: Structure-activity relationships of novel cannabinoid mimetics. *J Med Chem* 1995;38:3094–3105.
261. Haycock DA, Kuster JE, Stevenson JI, Ward SJ, D'Ambra T. Characterization of aminoalkylindole binding: Selective displacement by cannabinoids. *Probl. Drug Depend; Harris, L. s., Ed; NIDA Research Monograph No. 105, 1990; pp. 304–305.*
262. Rinaldi-Carmona M, Barth F, Héaulme M, Shire D, Calandra B, Congy C, Martinez S, Maruani J, Néliat G, Caput D, Ferrara P, Soubrié P, Brelière JC, Le Fur G. SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. *FEBS Lett* 1994;350:240–244.
263. Felder CC, Joyce KE, Briley EM, Glass M, Mackie KP, Fahey KJ, Cullinan GJ, Hunden DC, Johnson DW, Chaney MO, Koppel GA, Brownstein M. LY320135, a novel cannabinoid CB₁ receptor antagonist, unmasks coupling of the CB₁ receptor to stimulation of cAMP accumulation. *J Pharmacol Exp Ther* 1998;284:291–297.
264. Rinaldi-Carmona M, Barth F, Millan J, Derocq JM, Casellas P, Congy C, Oustric D, Sarran M, Bouaboula M, Calandra B, Portier M, Shire D, Brelière JC, Le Fur G. SR144528, the first potent and selective antagonist of the CB₂ cannabinoid receptor. *J Pharmacol Exp Ther* 1998;284:644–650.

265. Griffin G, Wray EJ, Tao Q, McAllister SD, Rorrer WK, Aung M, Martin BR, Abood ME. Evaluation of the cannabinoid CB₂ receptor-selective antagonist, 2 SR144528: Further evidence for cannabinoid CB₂ receptor absence in the rat central nervous system. *Eur J Pharmacol* 1999;377:117–125.
266. Panikashvili D, Simeonidou C, Ben-Shabat S, Hanuš L, Breuer A, Mechoulam R, Shohami E. An endogenous cannabinoid (2-AG) is neuroprotective after brain injury. *Nature* 2001;413:527–531.
267. Fernández-Ruiz J, González S, Romero J, Ramos JA. Cannabinoids in neurodegeneration and neuroprotection. In: Mechoulam R, editor. *Cannabinoids as therapeutics*. Basel, Boston, Berlin: Birkhäuser Verlag; 2005. pp. 79–109.
268. Mauler F, Mittendorf J, Horváth E, De Vry J. Characterization of the diarylether sulfonylester (-)-(R)-3-(2-hydroxymethylindanyl-4-oxy)phenyl-4,4,4-trifluoro-1-sulfonate (BAY 38-7271) as a potent cannabinoid receptor agonist with neuroprotective properties. *J Pharmacol Exp Ther* 2002;302:359–368.
269. De Vry J, Jentzsch KR. Discriminative stimulus effects of BAY 38-7271, a novel cannabinoid receptor agonist. *J Pharmacol Exp Ther* 2002;457:147–152.
270. Mauler F, Hinz V, Augstein KH, Fassbender M, Horvath E. Neuroprotective and brain edema-reducing efficacy of the novel cannabinoid receptor agonist BAY38-7271. *Brain Res* 2003;989:99–111.
271. Mauler F, Horváth E, De Vry J, Jäger R, Schwarz T, Sandmann S, Weinz C, Heinig R, Böttcher M. BAY 38-7271: A novel highly selective and highly potent cannabinoid receptor agonist for the treatment of traumatic brain injury. *CNS Drug Rev* 2003;9:343–358.
272. Pertwee R, Griffin G, Fernando S, Li X, Hill A, Makriyannis A. AM630, a competitive cannabinoid receptor antagonist. *Life Sci* 1995;56:1949–1955.
273. Hosohata K, Quock RM, Hosohata Y, Burkey TH, Makriyannis A, Consroe P, Roeske WR, Yamamura HI. AM630 is a competitive cannabinoid receptor antagonist in the guinea pig brain. *Life Sci* 1997;61: PL115–PL118.
274. Hosohata Y, Quock RM, Hosohata K, Makriyannis A, Consroe P, Roeske WR, Yamamura HI. AM630 antagonism of cannabinoid-stimulated [S-35]GTP gamma S binding in the mouse brain. *Eur J Pharmacol* 1997;321:R1–R3.
275. Gatley SJ, Lan R, Volkow ND, Pappas N, King P, Wong CT, Gifford AN, Pyatt B, Dewey SL, Makriyannis A. Imaging the brain marijuana receptor: Development of a radioligand that binds to cannabinoid CB₁ receptors in vivo. *J Neurochem* 1998;70:417–423.
276. Ibrahim MM, Deng H, Zvonok A. Activation of CB₂ cannabinoid receptors by AM1241 inhibits experimental neuropathic pain: Pain inhibition by receptors not present in the CNS. *Proc Natl Acad Sci* 2003;100:10529–10533.
277. Luk T, Jin W, Zvonok A, Lu D, Lin X-Z, Chavkin C, Makriyannis A, Mackie K. Identification of a potent and highly efficacious, yet slowly desensitizing CB₁ cannabinoid receptor agonist. *Br J Pharm* 2004;142:495–500.
278. Deng H, Gifford AN, Zvonok AM, Cui G, Li X, Fan P, Deschamps JR, Flippen-Anderson JL, Gatley SJ, Makriyannis A. Potent cannabinergic indole analogues as radioiodinatable brain imaging agents for the CB₁ cannabinoid receptor. *J Med Chem* 2005;48:6386–6392.
279. Stoit AR, Lange JH, Hartog AP, Ronken E, Tipker K, Stuivenberg HH, Dijksman JA, Wals HC, Kruse CG. Design, synthesis and biological activity of rigid cannabinoid CB₁ receptor antagonists. *Chem Pharm Bull* 2002;50:1109–1113.
280. Barth F. Cannabinoid receptor agonists and antagonists. *Exp Opin Ther Patents* 1998;8:301–313.
281. Gallant M, Duffresne C, Gareau Y, Guay D, Leblanc Y, Prasit P, Rochette C, Sawyer N, Slipetz DM, Tremblay N, Metters KM, Labelle M. New class of potent ligands for the human peripheral cannabinoid receptor. *Bioorg Med Chem Lett* 1996;6:2263–2268.
282. Mussinu JM, Ruiu S, Mule AC, Pau A, Carai MAM, Loriga G, Murineddu G, Pinna GA. Tricyclic pyrazoles. Part 1: Synthesis and biological evaluation of novel 1,4-dihydroindeno[1,2-c]pyrazol-based ligands for CB₁ and CB₂ cannabinoid receptors. *Bioorg Med Chem* 2003;11:251–263.
283. Lange JHM, Coolen HKAC, van Stuivenberg HH, Dijksman JAR, Herremans AHJ, Ronken E, Keizer HG, Tipker K, McCreary AC, Veerman W, Wals HC, Stork B, Verveer PC, den Hartog AP, de Jong NMJ, Adolfs TJP, Hoogendoorn J, Kruse CG. 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.
284. Hertzog DL. Recent advances in the cannabinoids. *Expert Opin Ther Patents* 2004;14:1435–1452.
285. Brain CT, Dziadulewicz EK, Hart TW. Preparation of quinazolinone derivatives as CB agonists. *Novartis AG: WO03066603* (2003).
286. Liu QI, Makriyannis A. Heteroindanes: A new class of potent cannabimimetic ligands. *Univ. Connecticut: WO03035005* (2003) *European Patent EP1448557*.

287. Kozłowski JA, Shankar BB, Shih N, Tong L. Cannabinoid receptor agonists. Schering Corp.: WO2004000807 (2004) European Patent EP1539693.
288. Kozłowski JA, Shih N, Lavey BJ, Rizvi RK, Shankar BB, Spitler JM, Tong L, Wolin R, Wong MK. Cannabinoid receptor ligands. Schering Corp.: WO2004014825 (2004) European Patent EP1539662.
289. Page D, Walpole C, Yang H. Preparation of benzimidazolecarboxamides as CB₂ receptor agonists for treating pain and other disorders. AstraZeneca AB: WO04035548 (2004).
290. Makriyannis A, Deng H. Novel cannabimimetic ligands. Univ. Connecticut: WO02058636 (2002) European Patent EP1361876.
291. Kai H, Murashi T, Tomida M. Preparation of 2-(aryl or heteroarylalkylimino)-1,3-thiazine derivatives having affinity for cannabinoid receptor of type 2 and medicinal compositions containing them. Japan: Shionogi & Co. Ltd; WO02072562, 2002, 191 pages.
292. Yasui K, Morioka Y, Hanasaki K. Antipruritics. Japan: Shionogi & Co. Ltd; WO03070277, 2003, 283 pages.
293. Tada Y, Iso Y, Hanasaki K. Preparation of pyridone derivatives having affinity for cannabinoid type 2 receptor. Japan: Shionogi & Co. Ltd; WO02053543, 2002, 307 pages.
294. Ferrarini PL, Calderone V, Cavallini T, Manera C, Saccomanni G, Pani L, Ruiu S, Gessa GL. Synthesis and biological evaluation of 1,8-naphthyridin-4(1H)-on-3-carboxamide derivatives as new ligands of cannabinoid receptor. *Bioorg Med Chem* 2004;12:1921–1933.
295. Huffman JW, Zengin G, Wu M-J, Lu J, Hynd G, Bushell K, Thompson ALS, Bushell S, Tartal C, Hurst DP, Reggio PH, Selley DE, Cassidy MP, Wiley JL, Martin BR. Structure-activity relationships for 1-alkyl-3-(1-naphthoyl)indoles at the cannabinoid CB₁ and CB₂ receptors: Steric and electronic effects of naphthoyl substituents. *New highly selective CB₂ receptor agonists*. *Bioorg Med Chem* 2005;13:89–112.
296. Gatley SJ, Gifford AN, Volkow ND, Lan RX, Makriyannis A. I-123-labeled AM251: A radioiodinated ligand which binds in vivo to mouse brain cannabinoid CB₁ receptors. *Eur J Pharmacol* 1996;307:331–338.
297. Gallant M, Dufresne C, Gareau Y, Guay D, Leblanc Y, Prasit P, Rochette C, Sawyer N, Slipetz DM, Tremblay N, Metters KM, Labelle M. New class of potent ligands for the human peripheral cannabinoid receptor. *Bioorg Med Chem Lett* 1996;6:2263–2268.
298. New DC, Wong YH. BML-190 and AM251 act as inverse agonists at the human cannabinoid CB₂ receptor: signalling via cAMP and inositol phosphates. *FEBS Lett* 2003;536:157–160.
299. Hurst DP, Lynch DL, Barnett-Norris J, Hyatt SM, Seltzman HH, Zhong M, Song ZH, Nie JJ, Lewis D, Reggio PH. N-(Piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (SR141716A) interaction with LYS 3.28(192) is crucial for its inverse agonism at the cannabinoid CB₁ receptor. *Mol Pharmacol* 2002;62:1274–1287.
300. Ruiu S, Pinna GA, Marchese G, Mussinu JM, Saba P, Tambaro S, Casti P, Vargiu R, Pani L. Synthesis and characterization of NESS 0327: A novel putative antagonist of the CB₁ cannabinoid receptor. *J Pharmacol Exp Ther* 2003;306:363–370.
301. Iwamura H, Suzuki H, Ueda Y, Kaya T, Inaba T. In vitro and in vivo pharmacological characterization of JTE-907, a novel selective ligand for cannabinoid CB₂ receptor. *J Pharmacol Exp Ther* 2001;296:420–425.
302. Brizzi A, Brizzi V, Cascio MG, Bisogno T, Sirianni R, Di Marzo V. Design, synthesis, and binding studies of new potent ligands of cannabinoid receptors. *J Med Chem* 2005;48:7343–7350.
303. Brizzi A, Cascio MG, Brizzi V, Bisogno T, Dinatolo MT, Martinelli A, Tuccinardi T, Di Marzo V. Design, synthesis, binding, and molecular modeling studies of new potent ligands of cannabinoid receptors. *Bioorg Med Chem* 2007;15:5406–5416.
304. Huffman JW, Szklennik PV, Almond A, Bushell K, Selley DE, He H, Cassidy MP, Wiley JL, Martin BR. 1-Pentyl-3-phenylacetylindoles, a new class of cannabimimetic indoles. *Bioorg Med Chem Lett* 2005;15:4110–4113.
305. Huffman JW, Padgett LW, Isherwood ML, Wiley JL, Martin BR. 1-Alkyl-2-aryl-4-(1-naphthoyl)pyrroles: New high affinity ligands for the cannabinoid CB₁ and CB₂ receptors. *Bioorg Med Chem Lett* 2006;16:5432–5435.
306. Carpino PA, Griffith DA, Sakya S, Dow RL, Black SC, Hadcock JR, Iredale PA, Scott DO, Fichtner MW, Rose CR, Day R, Dibruno J, Butler M, DeBartolo DB, Dutcher D, Gautreau D, Lizano JS, O'Connor RE, Sands MA, Kelly-Sullivan D, Ward KM. New bicyclic cannabinoid receptor-1 (CB₁-R) antagonists. *Bioorg Med Chem Lett* 2006;16:731–736.
307. Jagerovic N, Hernandez-Folgado L, Alkorta I, Goya P, Navarro M, Serrano A, Rodriguez de Fonseca F, Dannert MT, Alsasua A, Suardiaz M, Pascual D, Martin MI. Discovery of 5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-3-hexyl-1H-1,2,4-triazole, a novel in vivo cannabinoid antagonist containing a 1,2,4-triazole motif. *J Med Chem* 2004;47:2939–2942.

308. Pavon FJ, Bilbao A, Hernandez-Folgado L, Cippitelli A, Jagerovic N, Abellan G, Rodriguez-Franco MI, Serrano A, Macias M, Gomez R, Navarro M, Goya P, Rodriguez de Fonseca F. Antiobesity effects of the novel in vivo neutral cannabinoid receptor antagonist 5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-3-hexyl-1H-1,2,4-triazole - LH 21. *Neuropharmacology* 2006;51:358–366.
309. Silva VLM, Silva AMS, Pinto DCGA, Jagerovic N, Callado LF, Cavaleiro JAS, Elguero J. Synthesis and pharmacological evaluation of chlorinated N-Alkyl-3- and -5-(2-hydroxyphenyl)pyrazoles as CB₁ cannabinoid ligands. *Monatsh Chem* 2007;138:797–811.
310. Lavey BJ, Kozlowski JA, Hipkin RW, Gonsiorek W, Lundell DJ, Piwinski JJ, Narula S, Lunn CA. Triaryl bis-sulfones as a new class of cannabinoid CB₂ receptor inhibitors: Identification of a lead and initial SAR studies. *Bioorg Med Chem Lett* 2005;15:783–786.
311. Gonsiorek W, Hesk D, Chen S-C, Kinsley D, Fine JS, Jackson JV, Bober LA, Deno G, Bian H, Fossetta J, Lunn CA, Kozlowski JA, Lavey B, Piwinski J, Narula SK, Lundell Daniel J, Hipkin RW. Characterization of peripheral human cannabinoid receptor (hCB₂) expression and pharmacology using a novel radioligand, [³⁵S]Sch225336. *J Biol Chem* 2006;281:28143–28151.
312. Mussinu JM, Ruiu S, Mule AC, Pau A, Carai MAM, Loriga G, Murineddu G, Pinna GA. Tricyclic pyrazoles. Part 1: Synthesis and biological evaluation of novel 1,4-dihydroindeno[1,2-c]pyrazol-based ligands for CB₁ and CB₂ cannabinoid receptors. *Bioorg Med Chem* 2003;11:251–263.
313. Murineddu G, Rulu S, Mussinu JM, Loriga G, Grella GE, Caral MAM, Lazzari P, Pani L, Pinna GA. Tricyclic pyrazoles. Part 2: Synthesis and biological evaluation of novel 4,5-dihydro-1H-benzoglindazole-based ligands for cannabinoid receptors. *Bioorg Med Chem* 2005;13:3309–3320.
314. Murineddu G, Lazzari P, Ruiu S, Sanna A, Loriga G, Manca I, Falzoi M, Dessi C, Curzu MM, Chelucci G, Pani L, Pinna GA. Tricyclic pyrazoles. 4. Synthesis and biological evaluation of analogues of the robust and selective CB₂ cannabinoid ligand 1-(2',4'-dichlorophenyl)-6-methyl-N-piperidin-1-yl-1,4-dihydroindeno[1,2-c]pyrazole-3-carboxamide. *J Med Chem* 2006;49:7502–7512.
315. Ohta H, Ishizaka T, Yoshinaga M, Morita A, Tomishima Y, Toda Y, Saito S. Sulfonamide derivatives as new potent and selective CB₂ cannabinoid receptor agonists. *Bioorg Med Chem Lett* 2007;17:5133–5135.
316. Duarte CD, Tributino JLM, Lacerda DI, Martins MV, Alexandre-Moreira MS, Dutra F, Bechara EJH, De-Paula FS, Goulart MOF, Ferreira J, Calixto JB, Nunes MP, Bertho AL, Miranda ALP, Barreiro EJ, Fraga CAM. Synthesis, pharmacological evaluation and electrochemical studies of novel 6-nitro-3,4-methylenedioxyphenyl-N-acylhydrazones derivatives: Discovery of LASSBio-881, a new ligand of cannabinoid receptors. *Bioorg Med Chem* 2007;15:2421–2433.
317. Di Marzo M, Casapullo A, Bifulco G, Cimino P, Ligresti A, Di Marzo V, Riccio R, Gomez-Paloma L. Synthesis, conformational analysis and CB₁ binding affinity of hairpin-like anandamide pseudopeptide mimetics. *J Peptide Sci* 2006;12:575–591.
318. Horswill JG, Bali U, Shaaban S, Keily JF, Jeevaratnam P, Babbs AJ, Reynet C. PSNCBAM-1, a novel allosteric antagonist at cannabinoid CB₁ receptors with hypophagic effects in rats. *Br J Pharmacol* 2007;152:805–814.
319. Salamone JD, McLaughlin PJ, Sink K, Makriyannis A, Parker LA. Cannabinoid CB₁ receptor inverse agonists and neutral antagonists: Effects on food intake, food-reinforced behavior and food aversions. *Physiol Behav* 2007;91:383–388.
320. Chambers AP, Vemuri VK, Peng Y, Wood JAT, Olszewska T, Pittman QJ, Makriyannis A, Sharkey KA. A neutral CB₁ receptor antagonist reduces weight gain in rat. *Am J Physiol Regul Integr Comp Physiol* 2007;293:R2185–R2193.
321. Sink KS, McLaughlin PJ, Wood JAT, Brown C, Fan P, Vemuri VK, Pang Y, Olszewska T, Thakur GA, Makriyannis A, Parker LA, Salamone JD. The novel cannabinoid CB₁ receptor neutral antagonist AM4113 suppresses food intake and food-reinforced behavior but does not induce signs of nausea in rats. *Neuropsychopharmacology* 2008;33:946–955.
322. Yasuno F, Brown AK, Zoghbi SS, Krushinski JH, Chernet E, Tauscher J, Schaus JM, Phebus LA, Chesterfield AK, Felder CC, Gladding RL, Hong J, Halldin C, Pike VW, Innis RB. The PET radioligand [¹¹C]MePPEP binds reversibly and with high specific signal to cannabinoid CB₁ receptors in nonhuman primate brain. *Neuropharmacology* 2008;33:259–269.
323. Cohen S, Stillman RC, editors. *Therapeutic potential of marijuana*. New York: Plenum; 1976. p. 515.
324. Mechoulam R, editor. *Cannabinoids as therapeutic agents*. Boca Raton: CRC Press; 1986. 186 p.
325. Grotenhermen F, Russo E, editors. *Cannabis and cannabinoids: Pharmacology, toxicology, and therapeutic potential*. Binghamton: Haworth Press; 2002. p. 440.
326. Guy GW, Whittle BA, Robson PJ, editors. *The medicinal uses of cannabis and cannabinoids*. London: Pharmaceutical Press; 2004. p. 488.
327. Mechoulam R, editor. *Cannabinoids as therapeutics*. Basel: Birkhaeuser Verlag; 2005. p. 272.

328. Ben Amar M. Cannabinoids in medicine: A review of their therapeutic potential. *J Ethnopharmacol* 2006; 105:1–25.
329. Russo E, Guy GW. A tale of two cannabinoids: The therapeutic rationale for combining tetrahydrocannabinol and cannabidiol. *Med Hypothes* 2006;66:234–246.
330. Onaivi ES, Sugiura T, Di Marzo V, editors. *Endocannabinoids: The brain and body's marijuana and beyond*. Boca Raton: CRC Press; 2006. p. 555.
331. Di Marzo V, Bifulco M, De Petrocellis L. The endocannabinoid system and its therapeutic exploitation. *Nat Rev Drug Discov* 2004;3:771–784.
332. Grant I, Cahn BR. Cannabis and endocannabinoid modulators: Therapeutic promises and challenges. *Clin Neurosci Res* 2005;5:185–199.
333. Ramos JA, Gonzalez S, Sagredo O, Gomez-Ruiz M, Fernandez-Ruiz J. Therapeutic potential of the endocannabinoid system in the brain. *Mini Rev Med Chem* 2005;5:609–617.
334. Lambert DM, Fowler CJ. The endocannabinoid system: Drug targets, lead compounds, and potential therapeutic applications. *J Med Chem* 2005;48:5059–5087.
335. Fowler CJ, Holt S, Nilsson O, Jonsson K-O, Tiger G, Jacobsson SOP. The endocannabinoid signaling system: Pharmacological and therapeutic aspects. *Pharmacol Biochem Behav* 2005;81:248–262.
336. Paradisi A, Oddi S, Maccarrone M. The endocannabinoid system in ageing: A new target for drug development. *Curr Drug Targ* 2006;7:1539–1552.
337. Pacher P, Batkai S, Kunos G. The endocannabinoid system as an emerging target of pharmacotherapy. *Pharmacol Rev* 2006;58:389–462.
338. Tucci SA, Halford JCG, Harrold JA, Kirkham TC. Therapeutic potential of targeting the endocannabinoids: Implications for the treatment of obesity, metabolic syndrome, drug abuse and smoking cessation. *Curr Med Chem* 2006;13:2669–2680.
339. Manzanares J, Julian MD, Carrascosa A. Role of the cannabinoid system in pain control and therapeutic implications for the management of acute and chronic pain episodes. *Curr Neuropharmacol* 2006;4:239–257.
340. Muccioli GG. Blocking the cannabinoid receptors: Drug candidates and therapeutic promises. *Chem Biodiv* 2007;4:1805–1827.
341. Basavarajappa BS. The endocannabinoid signaling system: A potential target for next-generation therapeutics for alcoholism. *Mini Rev Med Chem* 2007;7:769–779.
342. Di Marzo V. The endocannabinoid system for the development of new drugs for spasticity. *Drug Future* 2007;32:341–351.
343. Lotersztajn S, Teixeira-Clerc F, Hezode C, Nhieu JTV, Deveaux V, Mallat A. Endocannabinoids: Therapeutic prospects in chronic liver diseases. *Gastroenterol Clin Biol* 2007;31:255–258.
344. Di Marzo V, Petrosino S. Endocannabinoids and the regulation of their levels in health and disease. *Curr Opin Lipidol* 2007;18:129–140.
345. Darmani NA. Antiemetic action of Δ^9 -tetrahydrocannabinol and synthetic cannabinoids in chemotherapy-induced nausea and vomiting. In: Onaivi ES, editor. *Biology of marijuana*. London: Taylor & Francis; 2002. pp. 356–389.
346. Vincent BJ, McQuiston DJ, Einhorn LH, Nagy CM, Brames MJ. Review of cannabinoids and their antiemetic effectiveness. *Drugs* 1983;25(Suppl 1):52–62.
347. Abrahamov A, Abrahamov A, Mechoulam R. An efficient new cannabinoid antiemetic in pediatric oncology. *Life Sci* 1995;56:2097–2102.
348. Mechoulam R, Hanuš L. The cannabinoids: An overview. Therapeutic implications in vomiting and nausea after cancer chemotherapy, in appetite promotion, in multiple sclerosis and in neuroprotection. *Pain Res Manage* 2001;6:67–73.
349. Hanuš L, Avraham Y, Ben-Shushan D, Zolotarev O, Berry EM, Mechoulam R. Short term fasting and prolonged semistarvation have opposite effects on 2-AG levels in mouse brain. *Brain Res* 2003;983:144–151.
350. Berry EM, Mechoulam R. Tetrahydrocannabinol and endocannabinoids in feeding and appetite. *Pharmacol Ther* 2002;95:185–190.
351. Kirkham TC. Endocannabinoids in the regulation of appetite and body weight. *Behav Pharmacol* 2005;16:297–313.
352. Fride E, Bregman T, Kirkham TC. Endocannabinoids and food intake: Newborn suckling and appetite regulation in adulthood. *Exp Biol Med* 2005;230:225–234.
353. Haney M, Gunderson EW, Rabkin J, Hart CL, Vosburg SK, Comer SD, Foltin RW. Dronabinol and marijuana in HIV-positive marijuana smokers: Caloric intake, mood, and sleep. *JAIDS* 2007;45:545–554.
354. Beal J, Flynn N. AIDS-associated anorexia. *J Physicians Assoc AIDS Care* 1995;2:19–22.

355. Osei-Hyiaman D. Endocannabinoid system in cancer cachexia. *Curr Opin Clin Nutr Metab Care* 2007;10:443–448.
356. Bellocchio L, Mancini G, Vicennati V, Pasquali R, Pagotto U. Cannabinoid receptors as therapeutic targets for obesity and metabolic diseases. *Curr Opin Pharmacol* 2006;6:586–591.
357. Chen J, Matias I, Dinh T, Lu T, Venezia S, Nieves A, Woodward DF, Di Marzo V. Finding of endocannabinoids in human eye tissues: Implications for glaucoma. *Biochem Biophys Res Commun* 2005;330:1062–1067.
358. Pate DW. Glaucoma and cannabinoids. In: Grotenhermen F, Russo E, editors. *Cannabis and cannabinoids*. Binghamton: Haworth Press; 2002. pp. 215–224.
359. Di Marzo V, Bifulco M, De Petrocellis L. Endocannabinoids and multiple sclerosis: A blessing from the ‘inner bliss’? *Trends Pharmacol Sci* 2000;21:195–197.
360. Pertwee RG. Cannabinoids and multiple sclerosis. *Mol Neurobiol* 2007;36:45–59.
361. Baker D, Jackson SJ, Pryce G. Cannabinoid control of neuroinflammation related to multiple sclerosis. *Br J Pharmacol* 2007;152:649–654.
362. Shohami E, Mechoulam R. Multiple sclerosis may disrupt endocannabinoid brain protection mechanism. *Proc Natl Acad Sci* 2006;103:6087–6088.
363. Di Marzo V. The endocannabinoid system for the development of new drugs for spasticity. *Drugs Future* 2007;32:341–351.
364. Pryce G, Baker D. Control of Spasticity in a Multiple Sclerosis Model is mediated by CB₁, not CB₂, Cannabinoid Receptors. *Br J Pharmacol* 2007;150:519–525.
365. Witting A, Chen L, Cudaback E, Straiker A, Walter L, Rickman B, Moller T, Brosnan C, Stella N. Experimental autoimmune encephalomyelitis disrupts endocannabinoid-mediated neuroprotection. *Proc Nat Acad Sci* 2006;103:6362–6367.
366. Anderson MC, Greene C. Suppressing unwanted memories by executive control. *Nature* 2001;410:131–134.
367. Lutz B. The endocannabinoid system and extinction learning. *Mol Neurobiol* 2007;36:92–101.
368. Varvel A, Wise LE, Niyuhire F, Cravatt BF, Lichtman AH. Inhibition of fatty-acid amide hydrolase accelerates acquisition and extinction rates in a spatial memory task. *Neuropsychopharmacology* 2007;32:1032–1041.
369. Bucherelli C, Baldi E, Mariottini C, Passani M, Blandina P. Aversive memory reactivation engages in the amygdala only some neurotransmitters involved in consolidation. *Learn Mem* 2006;13:426–430.
370. Carrier EJ, Patel S, Hillard CJ. Endocannabinoids in neuroimmunology and stress. *Curr Drug Targets CNS Neurol Disord* 2005;4:657–665.
371. Fride E, Suris R, Weidenfeld J, Mechoulam R. Differential response to acute and repeated stress in cannabinoid CB₁ receptor knockout newborn and adult mice. *Behav Pharmacol* 2005;16:431–440.
372. Chhatwal JP, Davis M, Maguschak KA, Ressler KJ. Enhancing cannabinoid neurotransmission augments the extinction of conditioned fear. *Neuropsychopharmacology* 2005;30:516–524.
373. Guindon J, Hohmann AG. Cannabinoid CB₂ receptors: A therapeutic target for the treatment of inflammatory and neuropathic pain. *Br J Pharmacol* 2008;153:319–334.
374. Vuong LAQ, Mitchell VA, Vaughan CW. Actions of N-arachidonyl-glycine in a rat neuropathic pain model. *Neuropharmacology* 2007;54:189–193.
375. Davis MP. Oral nabilone capsules in the treatment of chemotherapy-induced nausea and vomiting and pain. *Expert Opin Investig Drugs* 2008;17:85–95.
376. Guy G, Costa B. Cannabinoids for use in the treatment of neuropathic pain. (patent) *PCT Int Appl* 2007; WO 2007148094, 49 pages.
377. Cheng Y, Hitchcock SA. Targeting cannabinoid agonists for inflammatory and neuropathic pain. *Expert Opin Investig Drugs* 2007;16:951–965.
378. Guy G, Wright S, Robson P. A combination of cannabinoids for the treatment of peripheral neuropathic pain. *PCT Int Appl* 2007;53: WO 2007052013
379. Liu C, Walker JM. Effects of a cannabinoid agonist on spinal nociceptive neurons in a rodent model of neuropathic pain. *J Neurophysiol* 2006;96:2984–2994.
380. Slatkin NE. Cannabinoids in the treatment of chemotherapy -induced nausea and vomiting: Beyond prevention of acute emesis. *J Support Oncol* 2007;5(Suppl 3):1–9.
381. Beal J, Flynn N. AIDS-associated anorexia. *J Phys Assoc AIDS Care* 1995;2:19–22.
382. Ortar G, Moriello AS, Cascio MG, De Petrocellis L, Ligresti A, Morera E, Nalli M, Di Marzo V. New tetrazole-based selective anandamide uptake inhibitors. *Bioorg Med Chem Lett* 2008;18:2820–2824.
383. Martinez-Orgado J, Fernandez-Lopez D, Lizasoain I, Romero J. The seek of neuroprotection: Introducing cannabinoids. *Recent Patents CNS Drug Discov* 2007;2:131–139.

Lumír Ondřej Hanuš received his M.Sc. degree in analytical chemistry from Palacký University, Olomouc, Czechoslovakia in 1972. In 1974 he obtained his Ph.D. from Palacký University, Olomouc, Czechoslovakia. During 1971–1990 he taught and did research at Department of Hygiene and epidemiology, Medical Faculty, Palacký University, Olomouc, Czechoslovakia. In 1978–1979 as Research Associate, School of Pharmacy, University of Mississippi, studied cannabis and coca leaves. In 1984 became Candidate of Chemical Sciences in analytical chemistry from Palacký University, Olomouc, Czechoslovakia. From 1990 is with School of Pharmacy, Hebrew University in Jerusalem. From 1994 was Associate Professor in organic chemistry from Palacký University, Olomouc, Czechoslovakia and from 1995 Doctor of Sciences in pharmaceutical chemistry from Charles University in Prague, Czechoslovakia. During 1997–1998 worked at NIDDK, NIH in Bethesda on oleamide research and 2001–2002 was with NIAAA, NIH, Bethesda and studied importance of some endocannabinoids during ground squirrel hibernation. He works 37 years on Cannabis research, 17 years on endocannabinoids and personally isolated anandamide and several others endocannabinoids. Awards: 2005 Hanuš Medal (The Czech Chemical Society in Prague, Czech Republic) for the furthering creditable work in the fields of chemistry; 2006 Memorial Medal (Rector of the Palacký University in Olomouc, Czech Republic) to 50. Anniversary of revival and reopening of the University in Olomouc at the occasion of delivering 13th annual lecture to the honour of J. L. Fischer; 2007 Doctor honoris causa (Masaryk University, Brno, Czech Republic).