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

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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.
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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;

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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-arachidonoylethanolamine, 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 (CB_1) receptor, for pharmacologically identifying the compound. The day after isolating the compound to be named anandamide, its affinity (K_i) for the CB_1 receptor was determined to be 39.0 $\pm$ 5.0 nM. In this system, the K_i of Δ^9-THC, one of the psychoactive components of Cannabis, was determined to be 46.0 $\pm$ 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 Raphel Mechoulam to be N-arachidonoylethanolamine, who named it anadamide, 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 "garâru" 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 *gán-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 bhanga 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 bhanga (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 bhanga (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 bhanga (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 think 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, Rabin [40] 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 C_{21} 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 cannabinol (CBN, $C_{21}H_{26}O_2$) 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

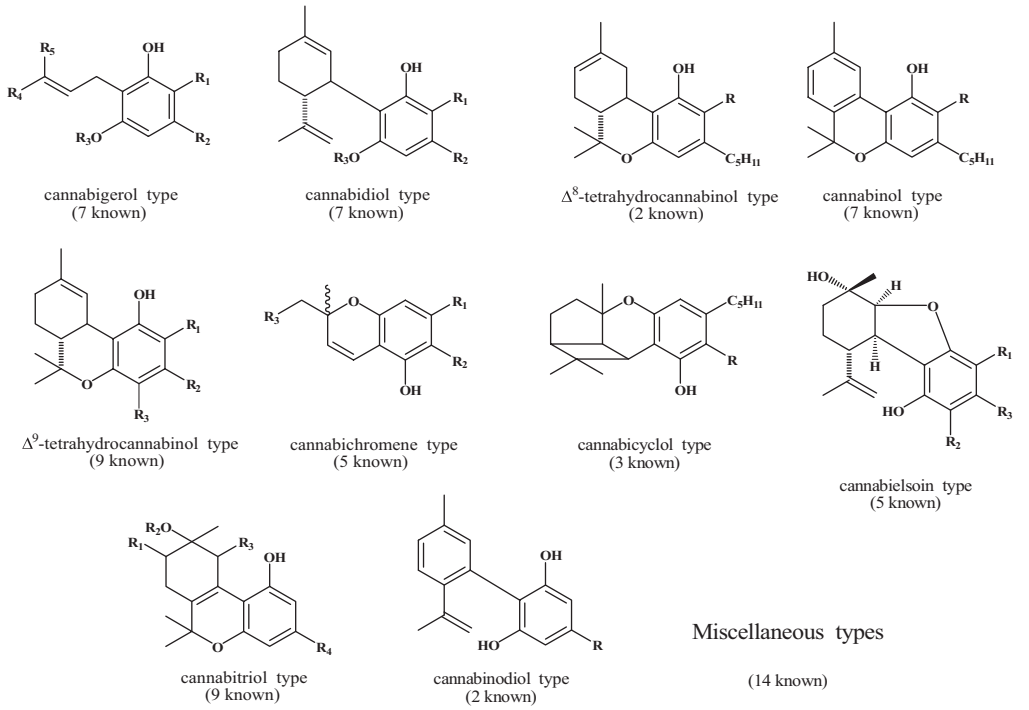


Figure 1. The main types of cannabinoids in *Cannabis sativa L.*

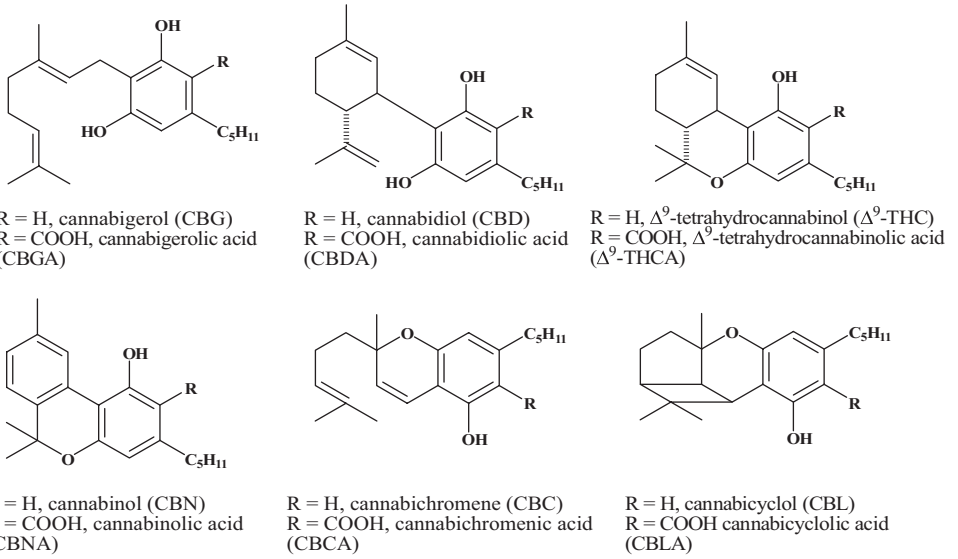


Figure 2. The main cannabinoids in *Cannabis sativa L.*

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.

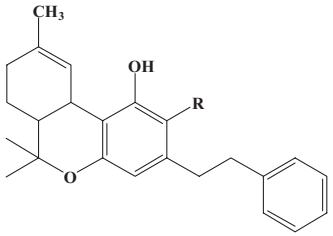
Advances in isolation methods made clarification of Cannabis chemistry possible. In 1963, Mechoulam's group isolated CBD and reported its correct structure and stereochemistry.[56] A year later they isolated pure tetrahydrocannabinol (Δ^9-THC), elucidated its structure, obtained a crystalline derivative 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 value[58] 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 Haffner[78] examined numerious 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 Todd[82] and Todd[83] 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 Farmilo[84] Building on Todd's work and using Birch's hypothesis[85] 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 Mechoulam[61] 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 extended[87] 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]

Razdan[88] 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 tetrahydrocannabitriol ester of CBDA.

De Faubert Maunder[89] 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 CBNA 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-^{14}C], sodium mevalonate-[2-^{14}C], geraniol-[1-^{3}H], nerol-[1-^{3}H], and CBGA-carboxyl-^{14}C, CBGA-[U-^{3}H] and CBDA-carboxyl-^{14}C, 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 cannabispirol and acetyl cannabispirol, in addition to cannabispirone 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 Japanese[93] but also Thai[94] hemp, including cannabispirenone, which gives rise to cannabispirone, cannabispirol and acetyl cannabispirol, and cannabispirenone, which could be converted to into cannabispirone and cannabispiranol. Cannabispiradienone 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 authors[97] 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 trichomes[98] and may be involved in the self-defense of the plant. THCA synthase has been cloned from the cDNA[99] 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 cannabispiradienone gives rise to cannabispirenone, further turning into cannabispirone and cannabispiranol. Cannabispiradienone is also source of cannabidihydrophenanthrene.

Turner and El-Sohly[107] 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 Itokawa[108] 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 Binder [110] 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.

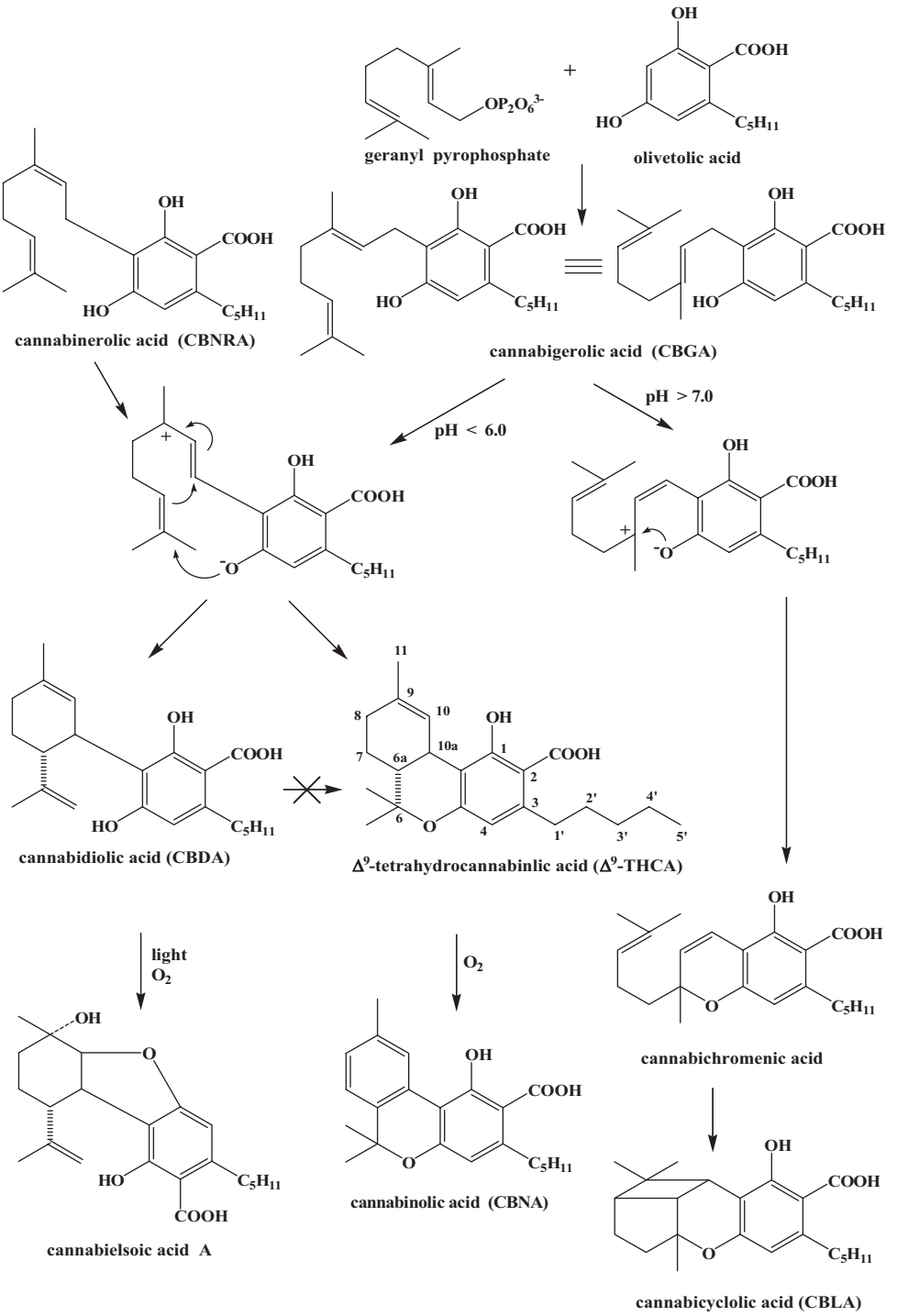


Figure 3. Proposed biogenesis of cannabinoid acids in the plant *Cannabis sativa L.*

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 cannabidivarol (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 C_3 and C_5 homologues.

Kajima and Piraux[114] utilized ^{14}C-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, Shoyama[115] 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-^{14}C (giving rise to tetrahydrocannabivarolic acid and cannabichromevarinic acid), cannabidivarolic acid-carboxyl-^{14}C (giving tetrahydrocannabivarolic acid), and cannabigerorcinic acid-carboxyl-^{14}C (producing two products, one of them identified as the cannabichromeorcinic 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 article[113] 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 (CB_1) and Peripheral (CB_2) 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 enantiomers[118]), specific for psychoactive cannabinoids, including Δ^8-THC[119] 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 CB_1. Homologues of this central cannabinoid receptor have been cloned from additional human[123,124] and mouse[125] tissues.

A second major cannabinoid was subsequently cloned from HL-60 cells.[126] This ''peripheral'' cannabinoid receptor, mCB_2, 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 CB_2 (hCB_2), but is shorter than

hCB$_2$ by 13 amino acids at the C-terminus. The gene for the rat CB$_2$ receptor was cloned, expressed, and its properties compared with those of mouse and human CB$_2$ receptors.[128] Sequence analysis of the rat CB$_2$ 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 CB$_2$ receptor resided exclusively in non-CNS tissues, Van Sickle et al.[129] reported the expression of CB$_2$ receptor mRNA and protein localization on brainstem neurons. It is now recognized that while the CB$_1$ receptor is found primarily in brain and neuronal tissue, and the CB$_2$ receptor is expressed primarily in immunocytes,[130] both the CB$_2$ receptor and its gene transcripts are widely distributed throughout the mammalian brain.[131,132] Both the CB$_1$ and CB$_2$ receptors are coupled through G proteins to adenylyl cyclase and mitogen-activated protein kinases. CB$_1$ 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 CB$_1$ in that it is not activated by other cannabinoids, but can be inhibited by the CB$_1$-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 ($EC_{50} = 18.4 \pm 4.1$ 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); Δ^9-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 CB$_1$, CB$_2$ 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 Pertwee[142] will be necessary to determine if GPR55 and its apparent endogenous ligands represent a new endogenous cannabinoid system.

5. ENDOCANNABINOIDS

The discovery of the CB$_1$ and CB$_2$ 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

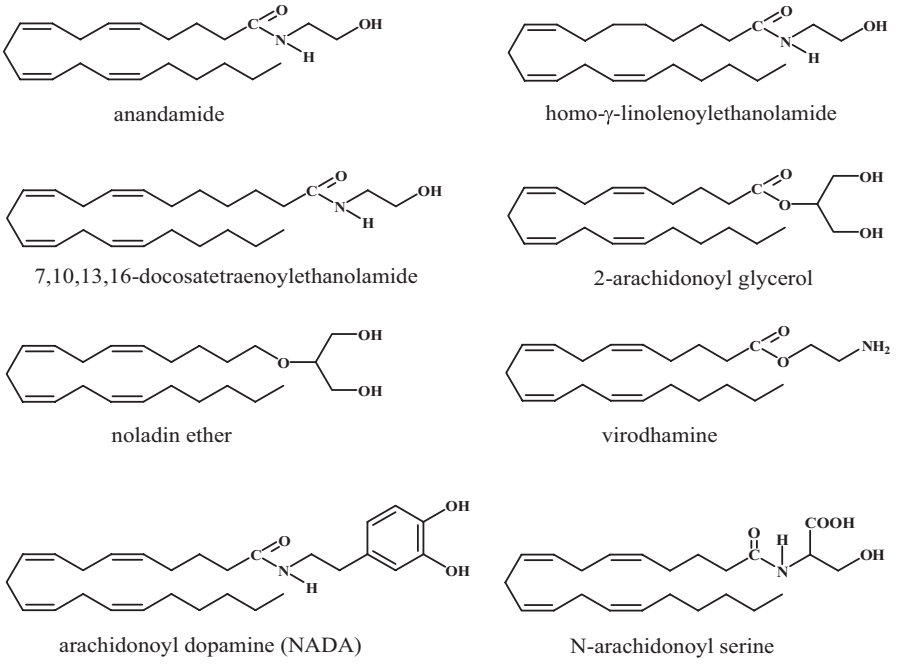


Figure 4. Known endocannabinoids.

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-linoleoylethanolamide (CB_1; $K_i = 53.4 \pm 5.5$ nM) and 7, 10, 13, 16-docosatetraenoylethanolamide (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; $K_i = 5.85 \pm 0.12$ μM).[147,148] The third major class of endocannabinoid is the ether-type, represented by 2-arachidonyl 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 ($K_i > 3$ μM). Administration of 2-arachidonyl glyceryl ether causes sedation, hypothermia, intestinal immobility, and mild antinociception in mice.[149]

Because of its structural similarity to anandamide, ARA-S[139] 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

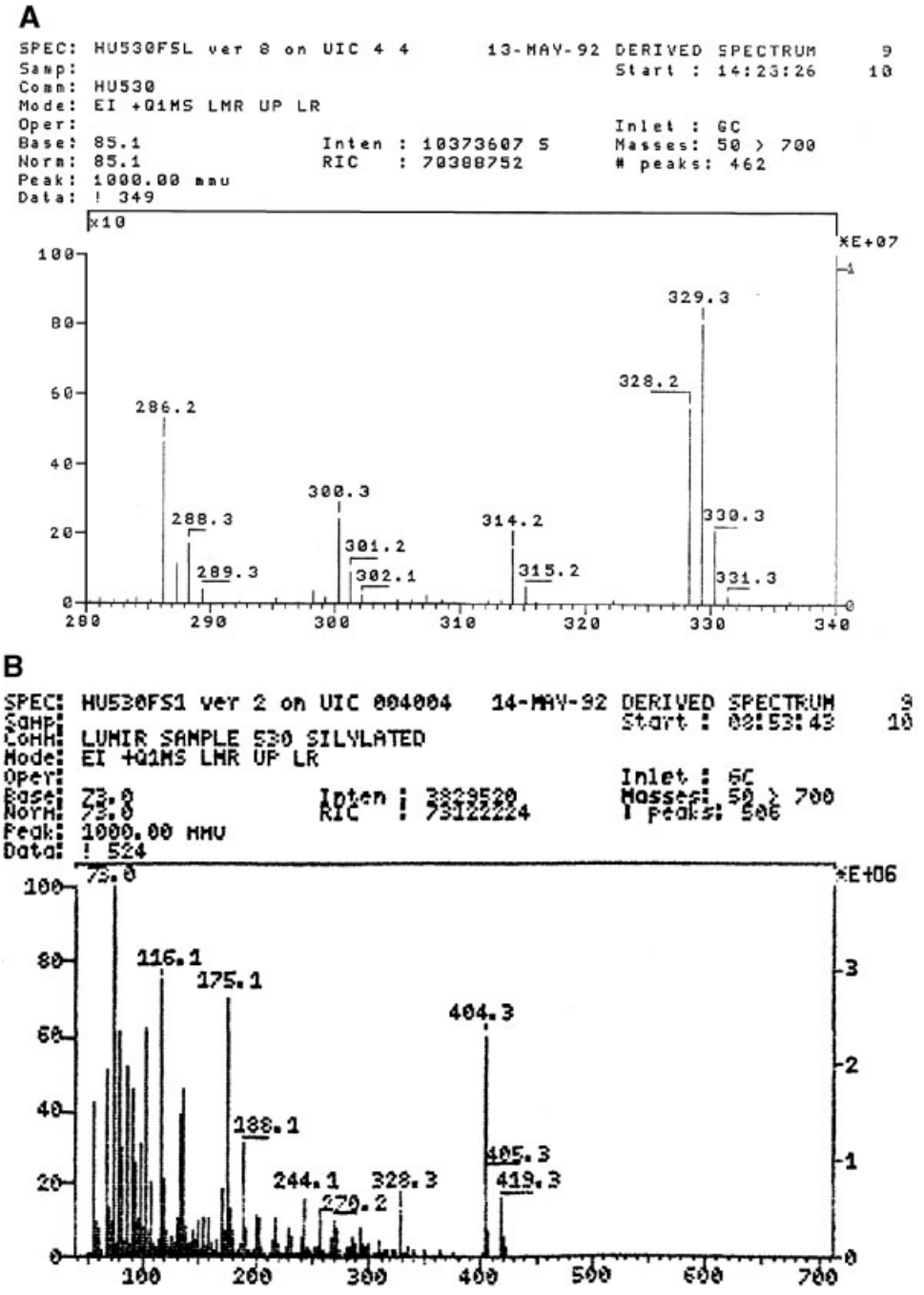


Figure 5. **A**: First known original mass spectrum of anandamide (HU-530) recorded by dr. Asher Gopher (May 13, 1992). **B**: First known original mass spectra of anandamide (HU-530) after silylation recorded by Dr. Asher Gopher (May 14, 1992).

in CB_1 or CB_2 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 CB_1 and CB_2 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 CB_1 receptor, with a 40-fold selectivity ($K_i = 250 \pm 130$ nM) over the CB_2 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

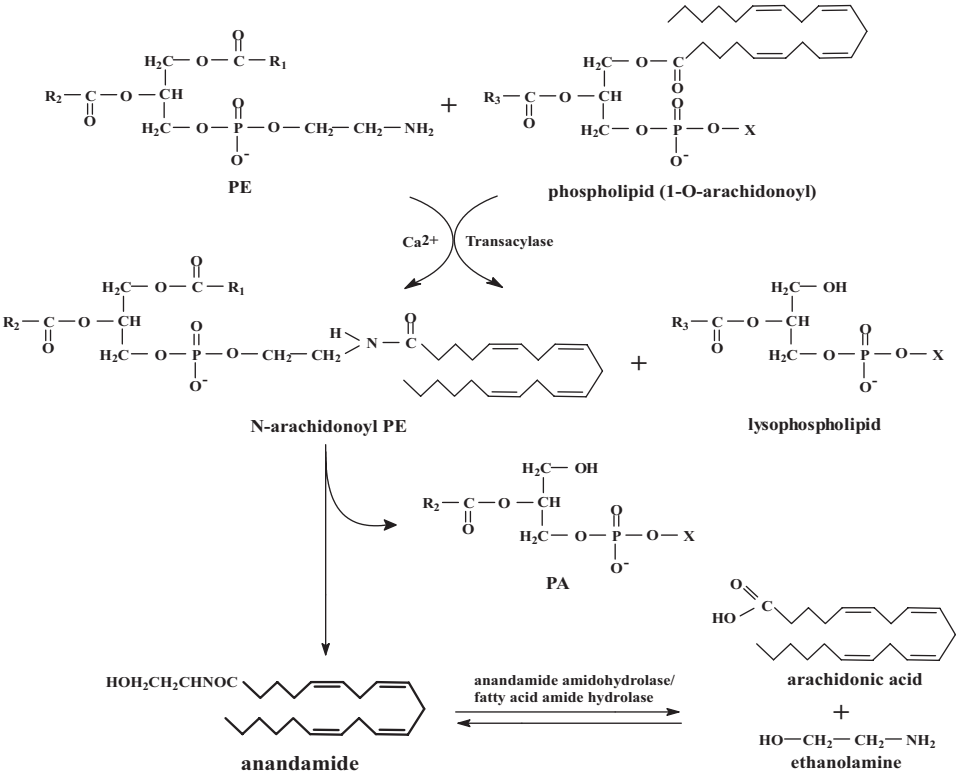


Figure 6. Pathways for the biosynthesis and degradation of anandamide.

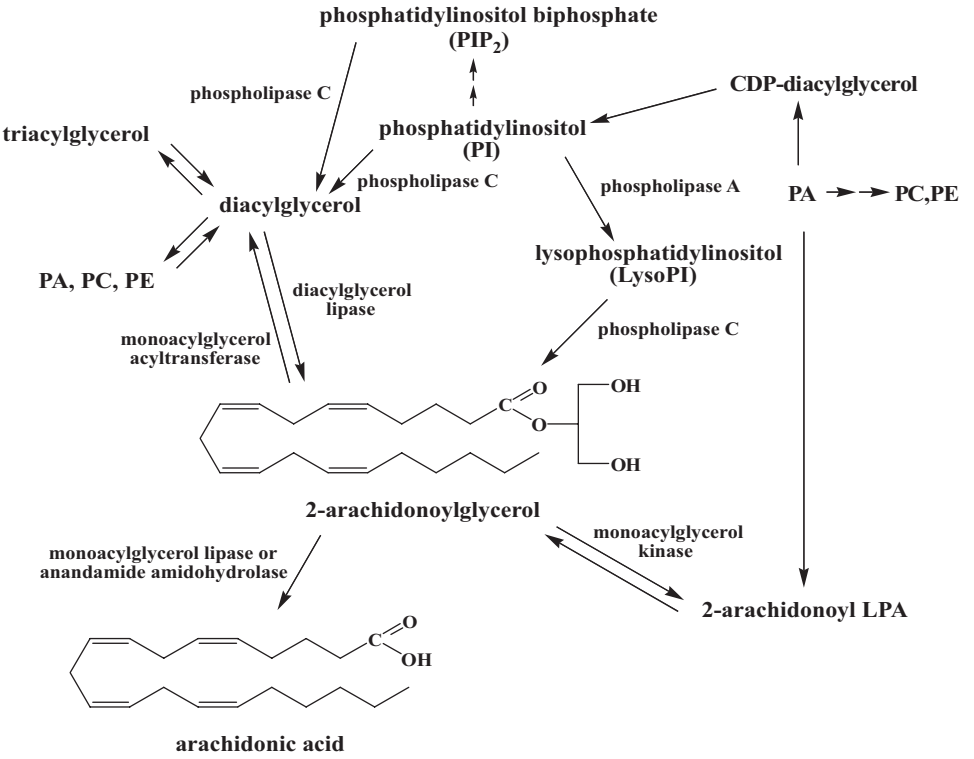


Figure 7. Pathways for the biosynthesis and degradation of 2-arachidonoylglycerol (2-AG).

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 precurosors, 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

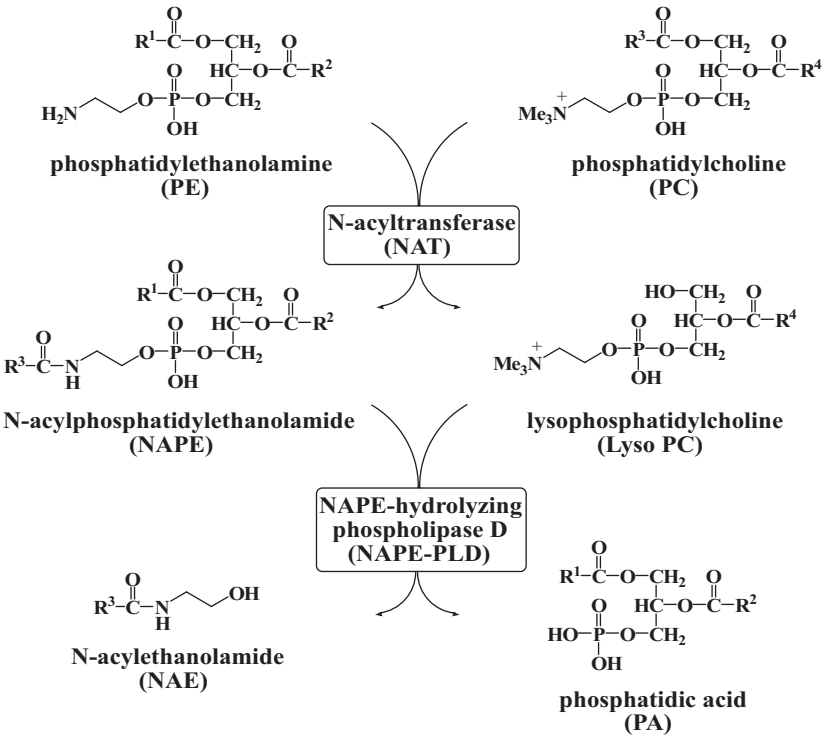


Figure 8. The biosynthesis of anandamide and other N-acylethanolamides via the transacylation-phosphodiesterase pathway.

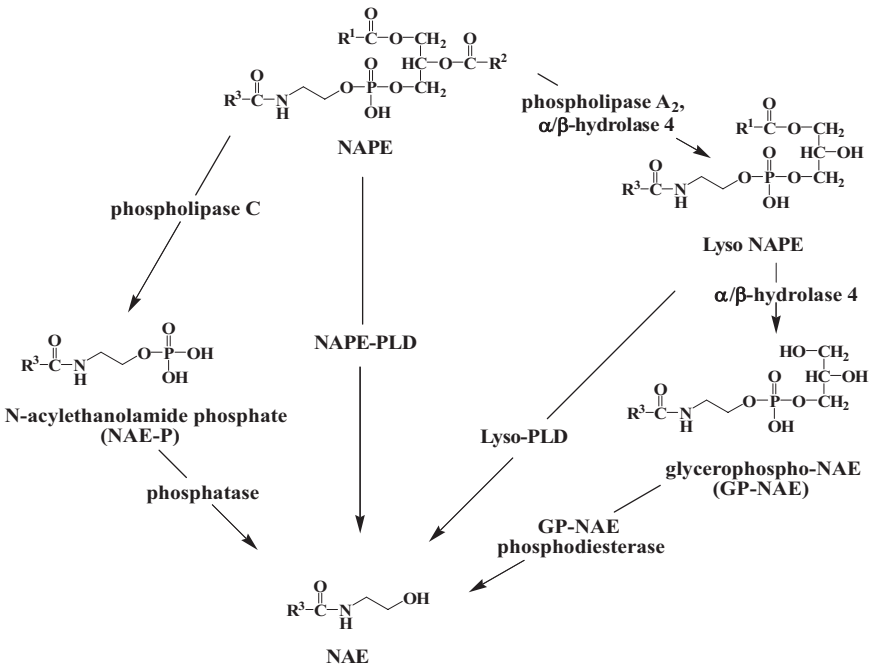
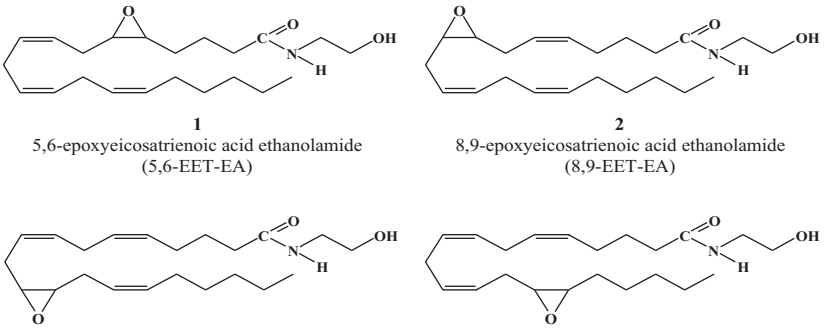


Figure 9. Multiple biosynthetic pathways involved in the formation of anandamide and other NAEs from NAPEs.

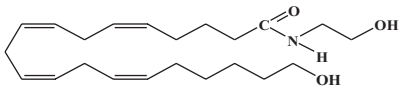


1
5,6-epoxyeicosatrienoic acid ethanolamide
(5,6-EET-EA)

2
8,9-epoxyeicosatrienoic acid ethanolamide
(8,9-EET-EA)

3
11,12-epoxyeicosatrienoic acid ethanolamide
(11,12-EET-EA)

4
14,15-epoxyeicosatrienoic acid ethanolamide
(14,15-EET-EA)



5
20-hydroxyeicosatetraenoic acid ethanolamide
(20-HETE-EA)

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 CB_1 and CB_2 receptors was studied.[172] Oxidative metabolism of endocannabinoids, anandamide and 2-AG (see Fig. 10), by

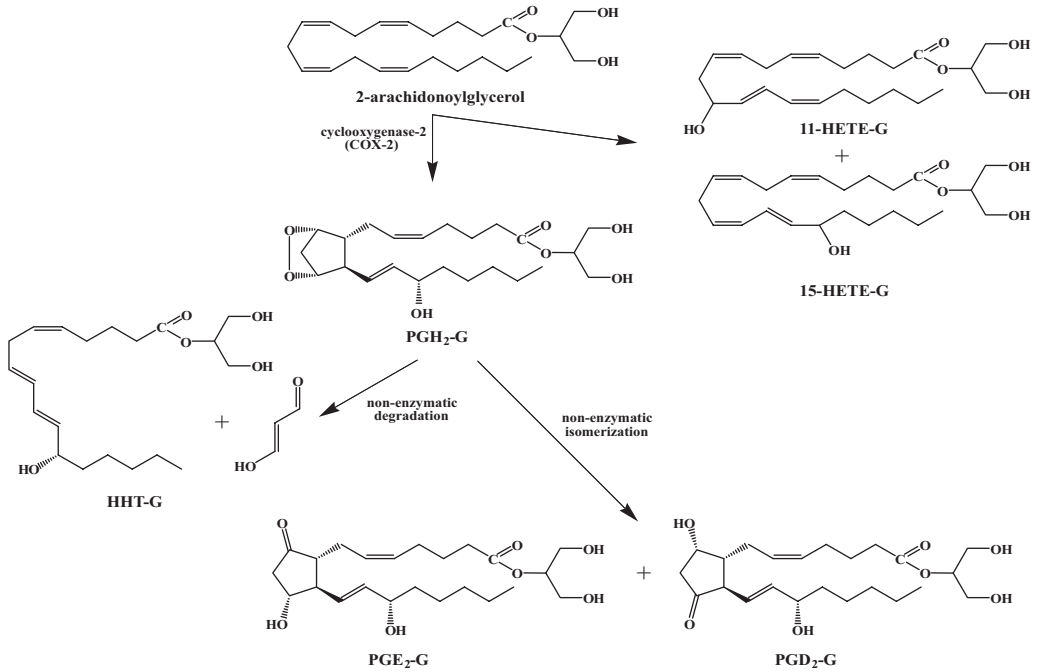


Figure 10. Oxidative metabolism of anandamide and 2-arachidonoylglycerol by cyclooxygenase-2.

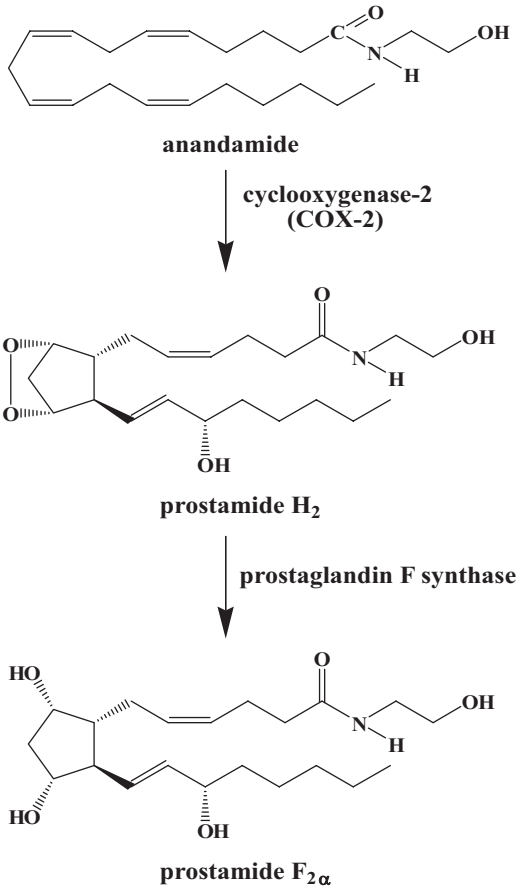


Figure 11. The biosynthesis of prostamide $F_{2\alpha}$ from anandamide.

cyclooxygenase-2 was studied intensively recently.[173–176] The biosynthesis of prostamide $F_{2\alpha}$ from anandamide was published.[177] Prostamide H_2 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 phophatidylcholine or phosphatidyl inositol 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 bellow).

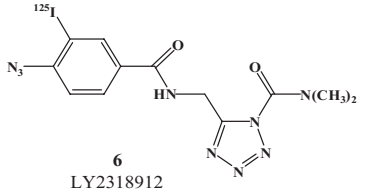
While the mechanisms of endocannabinoid inactivation are not completely understood, it appears to involve a two step process, where it is actively imported into cells where it is degraded by FAAH. A potent, competitive small molecule inhibitor of anandamide uptake (LY2318912, IC_{50} 7.27 $\pm$ 0.51 nM; **6**) was used to identify a high-affinity, saturable anandamide transporter binding site ($K_d = 7.62 \pm 1.18$ 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. Anadamide transporter inhibitors include *N*-(4-hydroxy-

phenyl)-arachidonoylethanolamide (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 hVR1.[183,184] These compounds only weakly inhibit anandamide hydrolysis and are poor CB_1/CB_2 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.

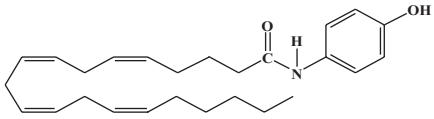
For a recent review on the cellular transport of endocannabinoids and its inhibition, see Fowler and Jacobsson.[187]

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]

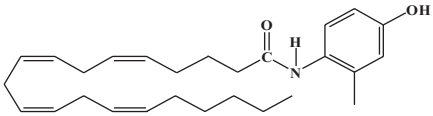
The first potent and relatively selective inhibitor of anandamide catabolism is methylarachidonylfluorophosphonate (MAFP, **12**).[191] MAFP and related analogs inhibit FAAH catalyzed hydrolysis of anandamide,[192] but also blocks arachidonyl ethanolamide amidase. Indeed, MAFP was found to irreversibly inhibit the CB_1 receptor, complicating interpretations of its activity.[193] In addition to FAAH, 2-AG is catabolised by monoacylglycerol lipase (MAGL). The presence of



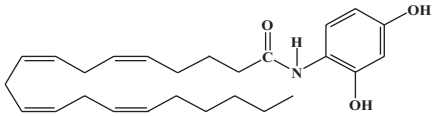
6 LY2318912



7 AM404



8 VDM11



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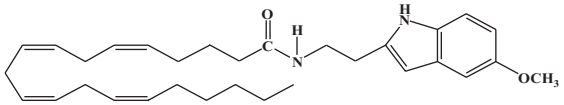

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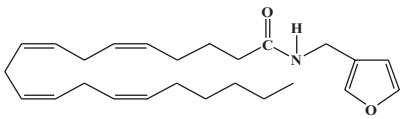
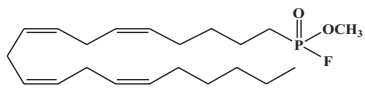
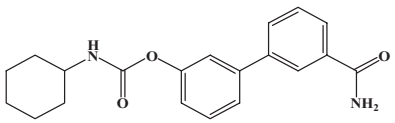


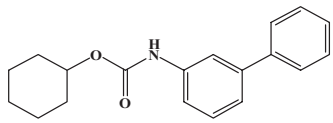
11 UCM707



12 MAFP



13 URB597



14 URB602

MAGL activity in microglial cells[194] 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 CB_1 or CB_2 receptors.[195–197] Palmitoylethanolamide binds to and potently activates ($EC_{50} = 3.2 \pm 1.3$ 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]

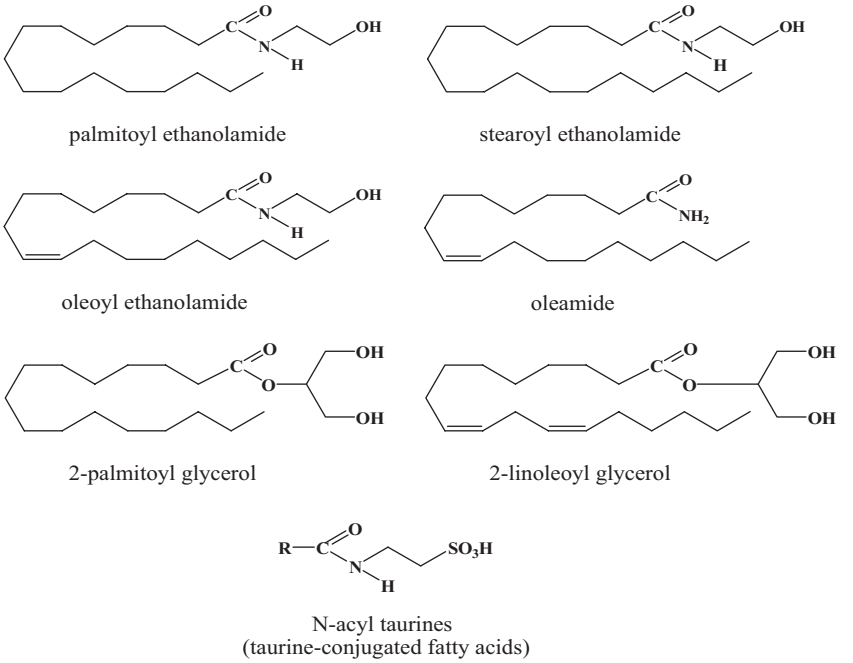


Figure 12. Known endocannabinoids congeners.

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 cats[205] and rats.[206,207] However, the mechanism by which oleamide induces sleep is poorly understood. It does not bind with high affinity to CB_1 or CB_2 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 CB_1 receptor agonist.[208] Oleamide relaxed small mesenteric arteries in the rat, an effect dependent on the presence of the endothelium, activation of Ca^{2+}-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]

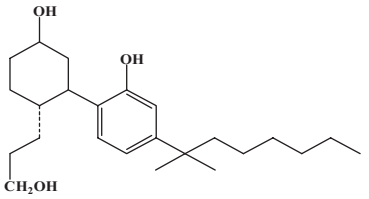
Several brain lipids are regulated by the mammalian enzyme fatty acid amide hydrolase *in vivo*, including a novel family of nervous system-enriched natural products, the taurine-conjugated fatty acids were discovered recently.[211] N-acyl taurines were found to activate multiple members of the transient 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

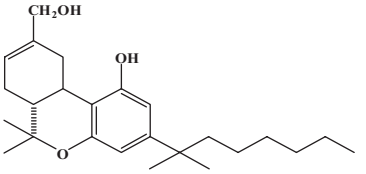
A. Synthetic Cannabinoid Analogues

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]

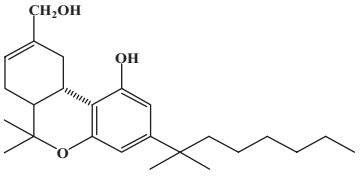
Subsequently, a number of agents were created with high affinity and stereospecificity.[218] Replacing the n-pentyl with a 1,1-dimethyl heptyl side chain of the primary active metabolite of Δ^8-THC, 11-hydroxy-Δ^8-THC, led to HU-210 (**16**) and its psychotropically inactive enantiomer, HU-211 (**17**). 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



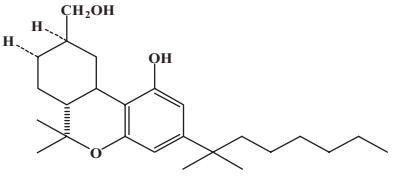
15 CP-55940



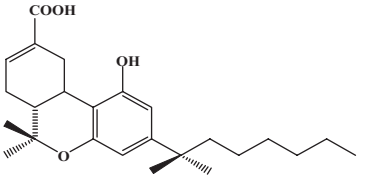
16 HU-210



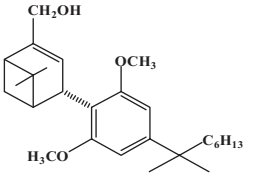
17 HU-211



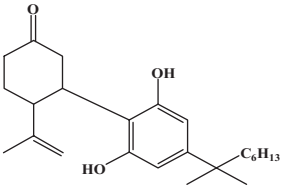
18 HU-243



19 ajulemic acid (CT_3, HU-239)



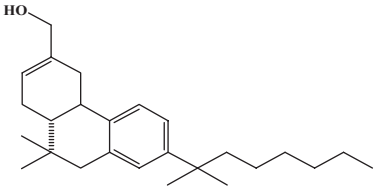
20 HU-308



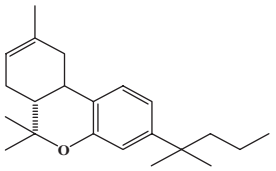
21 PRS 211,096

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 (CB_1, $K_i = 480.6$ nM in rat brain synaptosomal membranes; CB_2, $K_i = 170.5$ 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 (PPARγ)[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 CB_1 selective, the structurally related compound HU-308 (**20**) is CB_2-selective.[230] Both of the phenolic groups in HU-308 are blocked with methyl ethers, in contrast to cannabinoid CB_1 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 CB_1 receptor affinity. Other CB_2 receptor selective agents, including PRS-211096 (**21**), are being investigated for their immunomodulatory effects, including indications for the treatment of multiple sclerosis.[231]

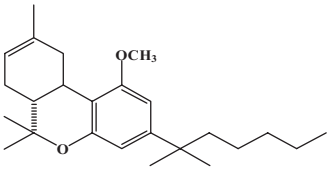
SAR have indicated that the cannabinoid side chain and the phenolic hydroxyl are key elements in CB_1 receptor recognition. To test this hypothesis, the 1-deoxy analogue, JWH-051 (**22**), of the very potent cannabinoid 11-hydroxy-Δ^8-THC-DMH (HU-210) was prepared and the affinity of this



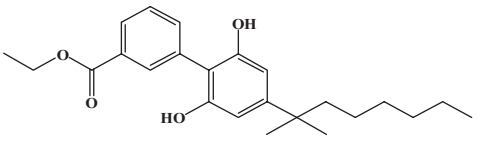
22 JWH-051



23 JWH-133



24 JWH-229



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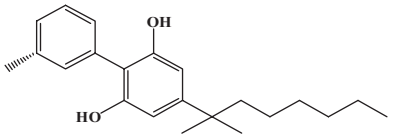


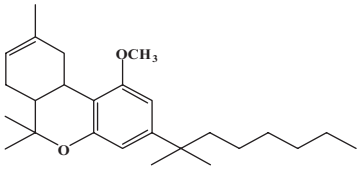
26

compound for the CB_1 receptor determined.[232] Contrary to expectations, this 1-deoxy analogue had high affinity for the CB_1 receptor ($K_i = 1.2 \pm 0.1$ nM) and even greater affinity for the CB_2 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 CB_2 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 CB_2 receptor, and four of which had low affinity ($K_i \geq 295$ nM) for the CB_1 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 CB_2 receptor ($K_i = 3.4 \pm 1.0$ nM) and low affinity for the CB_1 receptor ($K_i = 677 \pm 132$ nM).

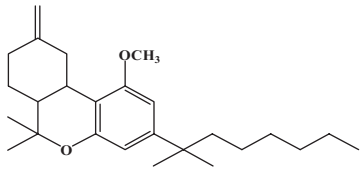
In view of the importance of the CB_2 receptor as a therapeutic target with reduced side-effect profile, three CB_2 selective cannabinoid receptor ligands, 1-methoxy-, 1-deoxy-11-hydroxy-, and 11-hydroxy-1-methoxy-Δ^8-tetrahydrocannabinols, were synthesized.[234] These compounds have greater affinity for the CB_2 receptor than for the CB_1 receptor, however only 1-methoxy-3-(1′, 1′-dimethylhexyl)-Δ^8-THC (JWH-229, **24**) had essentially no affinity for the CB_1 receptor ($K_i = 3{,}134 \pm 110$ nM) with high affinity for CB_2 ($K_i = 18 \pm 2$ nM). A series of biphenyls were synthesized as CB_2 selective modulators.[235] Compound **25** binds to CB_2 with a K_i value of 0.8 nM and to CB_1 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 CB_2 agonists.[236] Compound **26** had K_i values of 40 and 0.8 nM, respectively, for the CB_1 and CB_2 receptors. In addition, this compound was assessed by intravenous administration and exhibited ED_{50} values of 2.7, 2.4, and 3.6 mg/kg in the spontaneous activity, tail-flick and rectal temperature assays, respectively. CB_2 selective ligands, L759633 (**27**) and L759656 (**28**), that behave as potent, high-efficacy agonists, were characterized.[237]

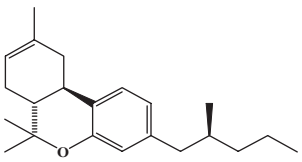
New series of cannabinoid ligands for the CB_2 receptor were prepared.[238] All prepared ligands have greater affinity for the CB_2 receptor then the CB_1 receptor. Four of these compounds, JWH-255 (**29**), JWH-352 (**30**), JWH-353 (**31**), and JWH-359 (**32**), have good activity for CB_2 receptor (24 nM,



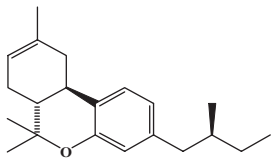
27 L759633



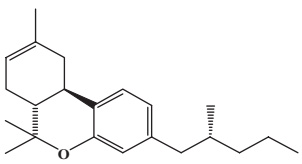
28 L759656



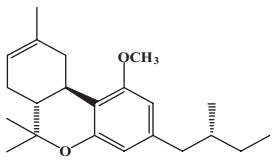
29 JWH-255



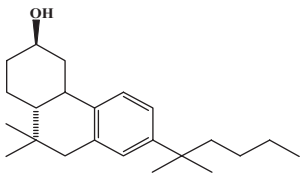
30 JWH-352



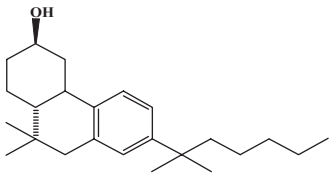
31 JWH-353



32 JWH-359



33 JWH-300



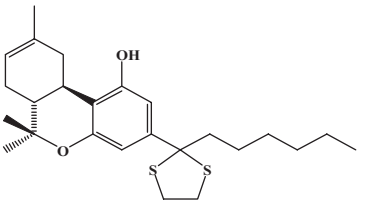
34 JWH-361

31 nM, 47 nM, and 13 nM) and weak affinity for the CB_1 receptor (4.3 μM, 1.5 μM, $>$ 10 μM, and 2.9 μM).

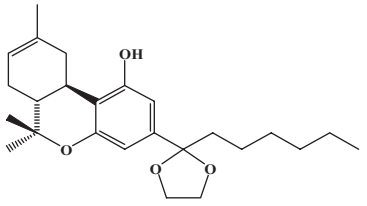
11-nor-1-methoxy-9-hydroxyhexahydrocannabinols and 11-nor-1-deoxy-9-hydroxyhexahydrocannabinols are new selective ligands for the CB_2 receptors.[239] From 14 prepared novel selective cannabinoids very high CB_2 affinity was determined for JWH-300 (**33**) (5.3 nM vs. 118 nM for CB_1 receptor) and JWH-361 (**34**) (2.7 nM against 63 nM for CB_1).

A successful attempt to conduct a molecular modeling and NMR-based 3D-QSAR CoMFA and CoMSIA studies of the CB_1 and CB_2 agonist pharmacophore models presented Durdagi et al.[240] A set of 30 novel Δ^8-THC and CBD analogues were studied. C1′-dithiolane (**35**) and C1′-dioxolane (**36**) analogues of Δ^8-THC showed high activity but low selectivity for the CB_1 ($K_i = 0.32$ respectively 0.52 nM) and CB_2 ($K_i = 0.52$ resp. 0.22 nM) receptors attributed to their fit in the hydrophobic subsite of both receptors. Δ^8-THC analogues had higher binding affinities than their respective CBD analogues.

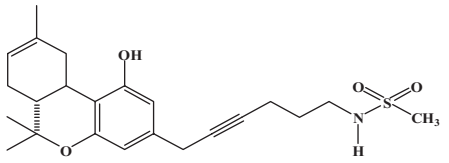
Martin et al.[241,242] prepared ''silent'' antagonist, O-2050 (**37**). This compound exhibited high CB_1 receptor affinity, but failed to produce either antinociception or hypothermia. Results of Gardner and Mallet[243] with this compound support the notion that cannabinoid receptor antagonists suppress feeding behavior by blocking an endogenous cannabinoid orexigenic signal, rather than by inverse agonism at cannabinoid receptors. Similar compound, O-2654 (6″-azidohex-2″-yne-cannabidiol, **38**), was prepared by Thomas et al.[244] This ligand is a competitive cannabinoid CB_1 receptor antagonist. Since it did not enhance the amplitude of electrically evoked contractions, it may be a neutral cannabinoid CB_1 receptor antagonist.



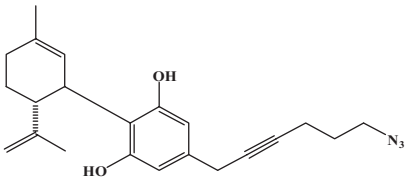
35 C1'-dithiolane analogue



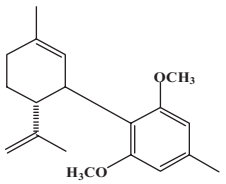
36 C1'-dioxolane analogue



37 O-2050



38 O-2654



39 O-1918

Finally neutral antagonist, O-1918 (**39**), prepared and studied Offertaler et al.[140] This cannabidiol analog does not bind to CB_1 or CB_2 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.

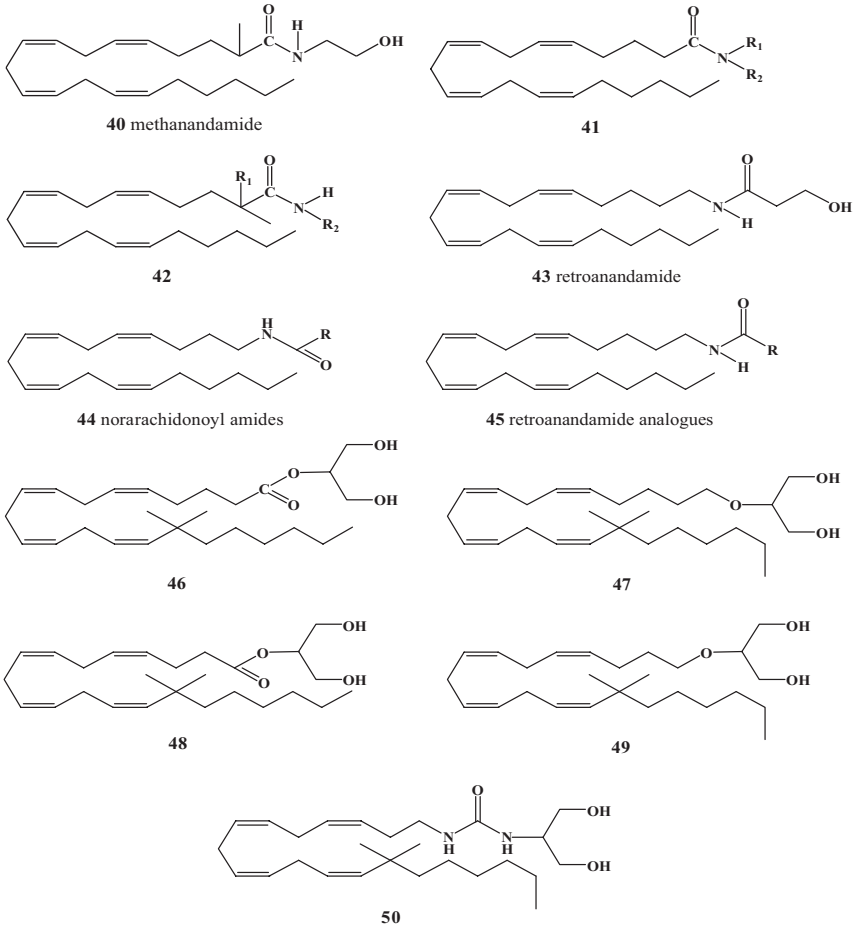
Several years ago a novel nonpsychoactive cannabinoid acid (HU-320) with antiinflamatory properties was synthetized.[245]

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 CB_1 than the CB_2 receptors, with K_i values of 20 ± 1.6 nM for the CB_1 receptor,[246] and 815 nM CB_2 receptor.[247] In order to establish the structural requirements for binding to the CB_1, 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 CB_1 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 CB_1 agonists, but more stable

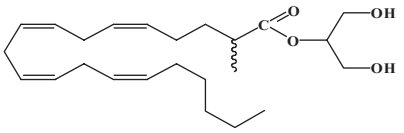


to degradation than 2-AG. The replacement of hydroxyl group by fluorine (**52a**, **52b**, **53**) did not improve the CB_1 activity. New synthetic substances were screened as DAGLα and MAGL inhibitors.[252] The novel compound, UP-101 (**54**) inhibited both DAGLα and MAGL with similar potencies. As the most potent inhibitors of DAGLα were O-3,640 (**55**), and O-384 (**56**).

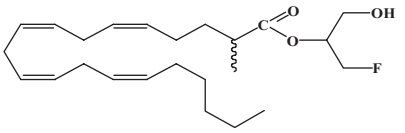
It was found recently,[253] that alkylamides A1 (**57**) and A2 (**58**) from Echinacea bind to the CB_2 receptor more strongly (the K_i values are for CB_2 receptor 57 and 60 nM, for CB_1 receptor 6.21 and 1.94 μM) than the endocannabinoid anandamide (for CB_2 receptor 218 nM and for CB_1 receptor 37 nM). All these compounds significantly inhibited lipopolysaccharide-induced tumor necrosis factor α, IL-1β, and IL-12p70 expression in a CB_2-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 CB_1 receptors and up to 20-fold CB_1/CB_2 selectivity—UP28 (**59**) (CB_1 200 nM, CB_2 4.0 μM), UP30 (**60**) (CB_1 800 nM, $CB_2 > 10$ μM), UP63 (**61**) (CB_1 200 nM, CB_2 4.0 μM), UP66 (**62**) (CB_1 150 nM, CB_2 4.3 μM), UP70 (**63**) (CB_1 300 nM, CB_2 5.1 μM), and UP27-18 (**64**) (CB_1 500 nM, $CB_2 > 10$ μM).

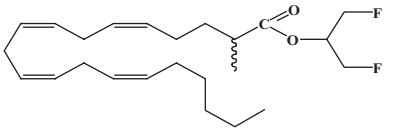
Two new potent and selective anandamide uptake inhibitors as potential antispastic drugs in multiple sclerosis were synthesized.[255] These compounds—O-3,246 (**65**) and O-3,262 (**66**)—are structural analogs of previously reported selective inhibitor (O-2,093, **67**) of the reuptake of anandamide.



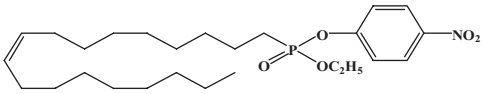
51a (R)
51b (S)



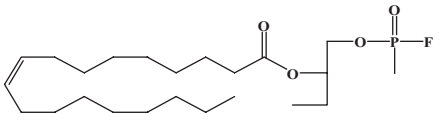
52a (R)
52b (S)



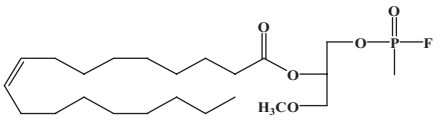
53 (R)



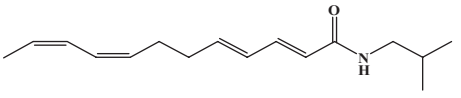
54 UP101



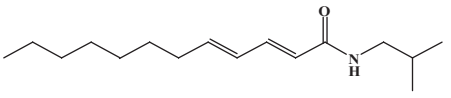
55 O-3640



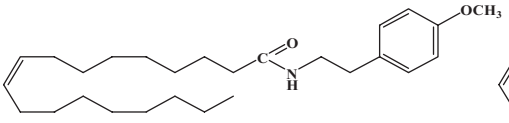
56 O-3841



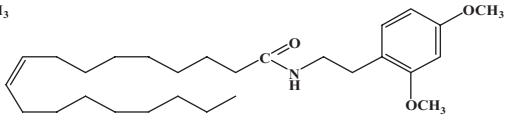
57 A1



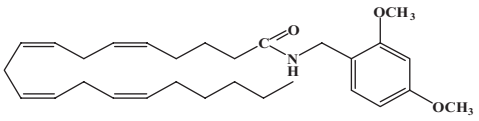
58 A2



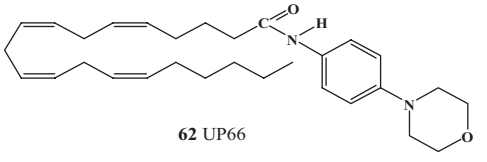
59 UP28



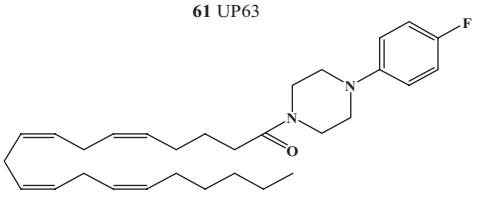
60 UP30



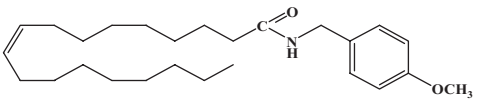
61 UP63



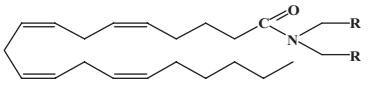
62 UP66

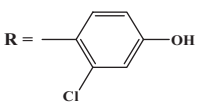


63 UP70

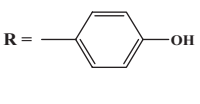


64 UP27-18

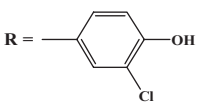




65 O-3246

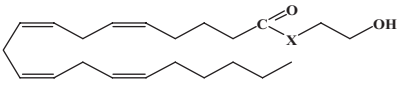


66 O-3262

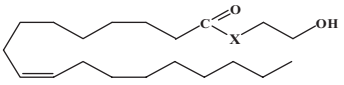


67 O-2093

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 CB_2 receptor (K_i for CB_1 = 470 nM, for CB_2 = 81 nM).



68a, X = -NH- **68c**, X = -N(OH)-
68b, X = -NH-O- **68d**, X = -O-NH-



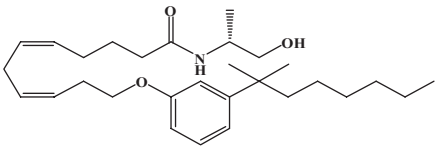
69a, X = -NH- **69c**, X = -N(OH)-
69b, X = -NH-O- **69d**, X = -O-NH-

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 CB_1 receptors, but also to CB_2 ones.

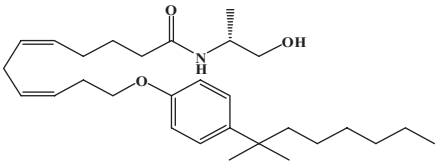
C. Synthetic Noncannabinoid Ligands

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]

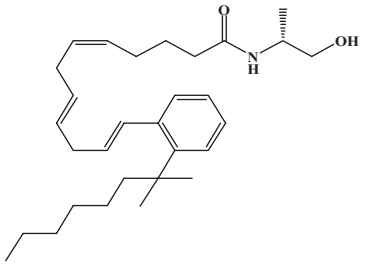
Shortly thereafter, the first potent and selective CB_1 antagonist was created, SR-141716A (**80**).[262] Followed by the CB_1 antagonist, LY320135 (**81**), which is not as selective as the previous



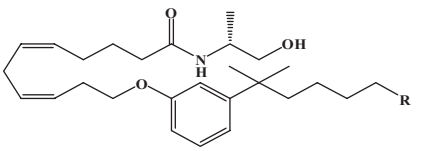
70, O-2220



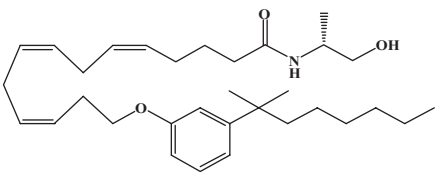
71, O-2294



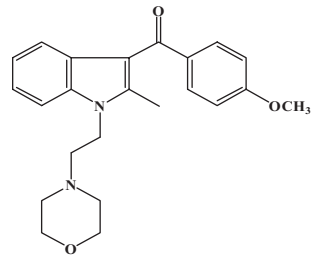
72, O-2243



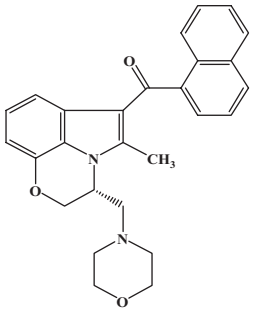
73, R = OH; O-2760
74, R = Br; O-2874
75, R = N_3; O-2781
76, R = CN; O-2852



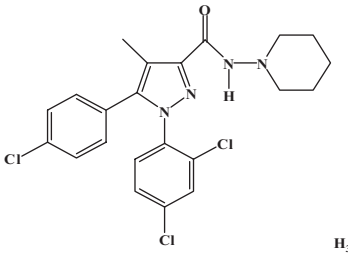
77, O-2655



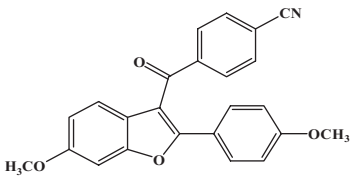
78 Pravadoline



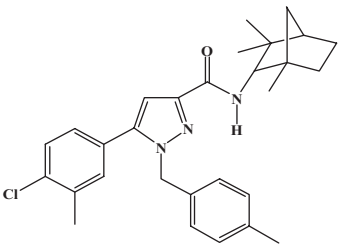
79 WIN 55212-2



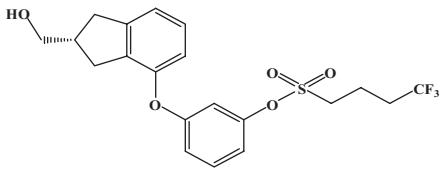
80 SR 141716A (rimonabant)



81 LY320135



82 SR144528



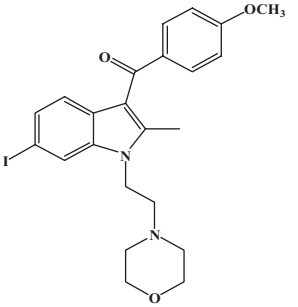
83 BAY 38-7271

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

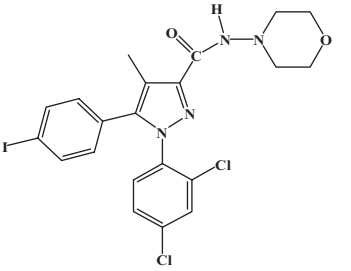
The Sanofi group also described the first potent and selective antagonist of the peripheral cannabinoid receptor (CB_2), SR 144528 (**82**).[264,265]

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 CB_1/CB_2 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]

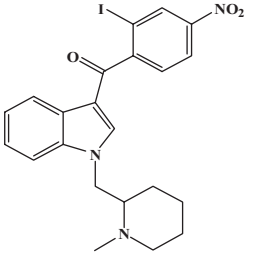
6-Iodo-pravadoline (AM-630, **84**), an aminoalkylindole, attenuates the ability of a number of cannabinoids to inhibit electrically evoked twitches of the isolated mouse vas deferens.[272] AM-630 behaves also as a competitive antagonist of cannabinoid receptor agonists in the guinea pig brain.[273]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



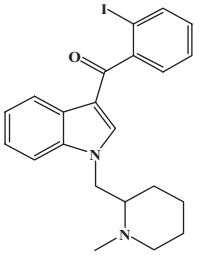
84 AM-630



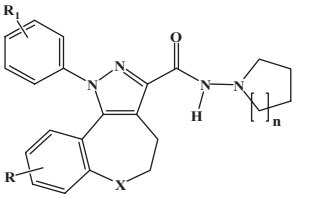
85 AM 281



86 AM 1241



87 AM 2233



88 pyrazole-based tricyclic CB_1 antagonists

Gatley et al. have developed a novel radioligand, [^{123}I]AM-281 (**85**), structurally related to the CB_1 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 CB_2 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 CB_2 receptor in a mouse spleen preparation ($K_i = 3.4 \pm 0.5$ nM), with good selectivity *versus* the CB_1 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 CB_1 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 CB_1 receptor. AM-2233 represents the first agonist CB_1 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 CB_1 receptor antagonists (**88**).[279] Additional information on cannabinoid receptor agonists and antagonists is reported in Barth's review.[280]

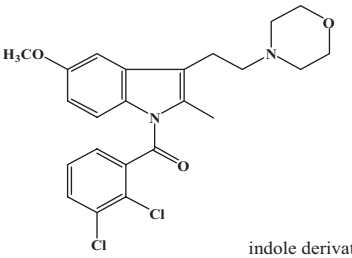
Gallant et al.[281] have described two indole-derived compounds (**89**, **90**), with binding potency for the human peripheral cannabinoid receptor (hCB_2) in the nanomolar region. They are highly selective.

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* CB_2 binding affinities. Four compounds showed very high selectivity for the CB_2 receptor.

Recently the discovery of a further class of diarylpyrazolines (**92**) with high potency and selectivity for the CB_1 receptor was described.[283] These compounds were found to be CB_1 antagonists. SLV319 (**93**) was found to be a potent CB_1 antagonist ($K_i = 7.8$ nM) close to that of the Sanofi compound SR141716A with more than 1,000-fold selectivity against CB_2.

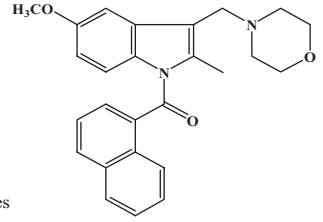
Additional synthetic compounds, which bind to the CB_1 and/or CB_2 receptors, have been mentioned in patents. These were recently reviewed by Hertzog.[284]

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 CB_1 ($K_i = 34$ nM) and CB_2 ($K_i = 11$ nM). The patent application refers to the compound as having CB_2 agonist activity. Additionally, this

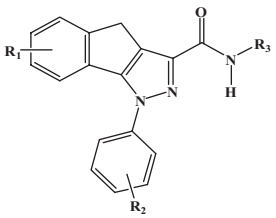


indole derivatives

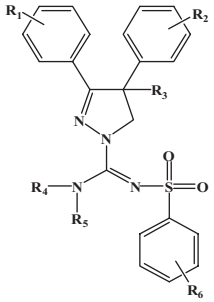
89



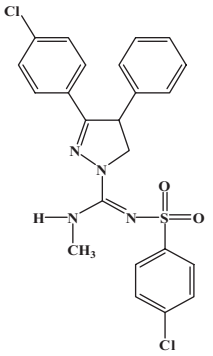
90



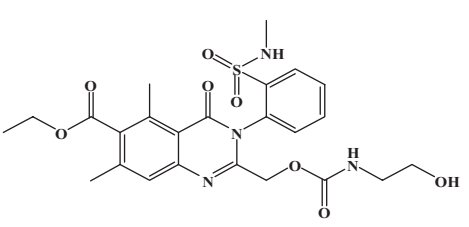
91 1,4-dihydroindeno[1,2-c]pyrazole derivatives



92 diarylpyrazolines



93 (-)-enantiomer (SLV319)



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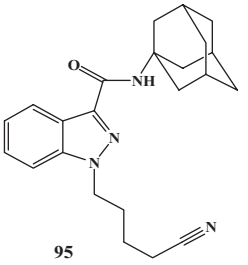

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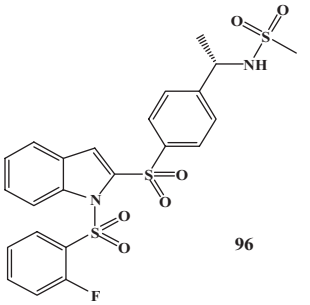

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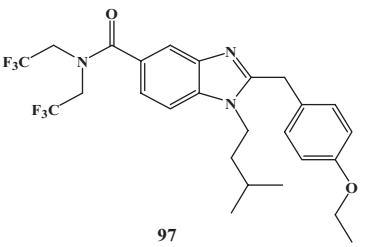


97

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 CB_2 over CB_1. Compound **95**, for instance, exhibited K_i values of 2.28 and 0.309 nM for the CB_1 and CB_2 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 CB_1 receptor.

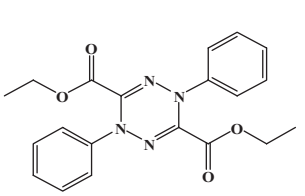
A series of aromatic CB_2 agonists has been disclosed by Schering-Plough Research Institute.[287,288] The compounds are reported to have anti-inflammatory and immunomodulatory activities, and to be active in cutaneous T cell lymphoma, diabetes mellitus and other indications. Compound **96** is stated to bind to CB_2 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 CB_2 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 CB_2 ($K_i = 3.1$ nM) with much greater affinity than to CB_1 ($K_i = 2.8$ μM). No *in vivo* data are provided for the compounds.

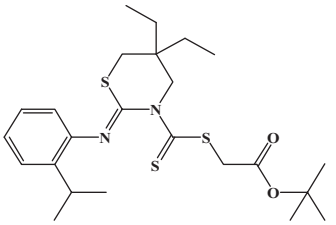
The University of Connecticut has disclosed a series of dihydrotetrazines and derivatives as CB_2 agonists.[290] Compound **98** is reported to be a potent CB_2 agonist ($K_i = 19$ nM) with 88-fold selectivity for the CB_2 over the CB_1 receptor. Such compounds are reported to be useful in the treatment of pain, glaucoma, multiple sclerosis, Parkinson's disease, Alzheimer's disease, and other disorders.

Shionogi has also disclosed two series of thiazine-containing CB_2 agonists, of which compounds **99** and **100** are examples.[291,292] Selectivity data for several of the compounds with regard to CB_2:CB_1 affinities are described. For example, compounds **90** binds to CB_2 with a K_i value of 0.3 nM and a K_i value of >5,000 nM for CB_1. Compound **91** displayed a K_i value of 1.2 nM at the CB_2 receptor and 80 nM at the CB_1 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 CB_2 modulators stated to be useful in the treatment of inflammation, nephritis, pain, allergies, rheumatoid arthritis, multiple sclerosis, brain



98



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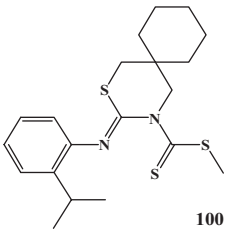

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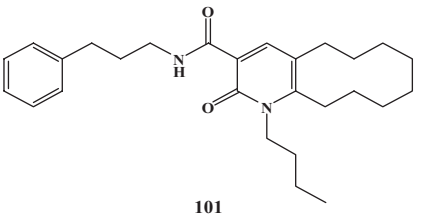

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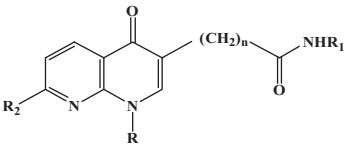

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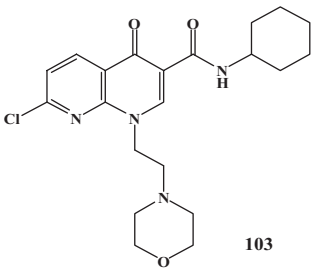


103

tumors, and glaucoma.[293] Compound **101** was found to bind to the CB_2 receptor with a K_i value of 4 nM, with very little affinity for CB_1 ($K_i < 5$ μM).

Recently 1,8-naphthyridin-4(1*H*)-on-3-carboxamide derivatives (**102**) as new ligands of cannabinoid receptors were synthesized.[294] Some of these compound possess a greater affinity for the CB_2 receptor than for the CB_1 receptor. Compound 7-chloro-*N*-cyclohexyl-1-(2-morpholin-4-ylethyl)-1,8-naphthyridin-4(1*H*)-on-3-carboxamide (**103**) revealed a good CB_2 selectivity (CB_1, $K_i = 1$ μM; CB_2, $K_i = 25 \pm 1.8$ nM).

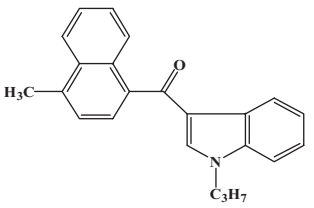
Indole derivatives were prepared and tested for their CB_1 and CB_2 receptor affinities.[295] Three new, highly selective CB_2 receptor agonists were identified, namely JWH-120 (**104**) (CB_1, $K_i = 1{,}054 \pm 31$ nM; CB_2, $K_i = 6.1 \pm 0.7$ nM), JWH-151 (**105**) (CB_1, $K_i > 10{,}000$ nM; CB_2, $K_i = 30 \pm 1.1$ nM) and JWH-267 (**106**) (CB_1, $K_i = 381 \pm 16$ nM; CB_2, $K_i = 7.2 \pm 0.14$ nM).

AM251 (**107**), an analog of the cannabinoid receptor antagonist SR141716A, has been shown to be a cannabinoid CB_1 receptor selective antagonist.[296]

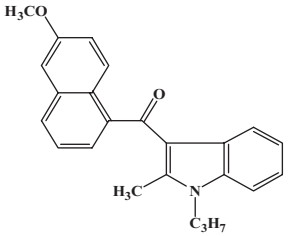
In earlier reports several interesting potent ligands were described. New potent ligands, BML-190 (**108**), L-768242 (**109**), and L-759787 (**110**), for the human peripheral cannabinoid receptor were synthesized.[297] Results of New and Wong[298] demonstrate that BML-190 and AM251 (cannabinoid antagonist and inverse agonist) behave as inverse agonists at the human CB_2 receptor acting *via* $G\alpha_{i/o}$ and $G\alpha_q$ family-coupled pathways.

Hurst et al.[299] designed and synthetized an SR141716A analog, called VCHSR (**111**). This ligand acted as a neutral antagonist at wild-type CB_1. Ruiu et al.[300] synthesized another putative cannabinoid ligand, antagonist NESS 0327 (**112**), with high selectivity for the cannabinoid CB_1 receptor.

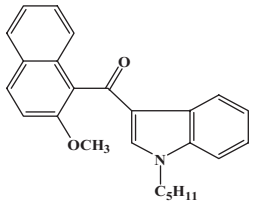
A novel selective ligand, JTE907 (**113**) was reported as an inverse agonist (binding affinity for rat CB_1 was 1.05 μM and for CB_2 0.38 nM, respectively).[301] This compound has antiedema effects *in vivo*.



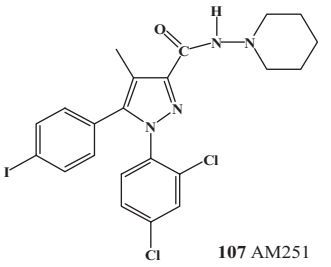
104 JWH-120



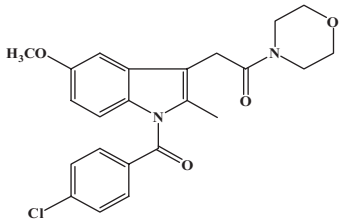
105 JWH-151



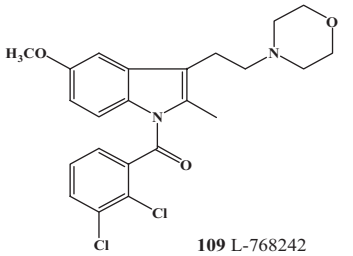
106 JWH-267



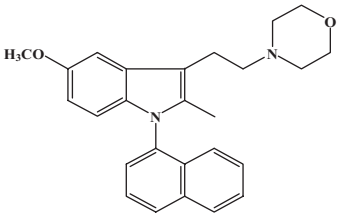
107 AM251



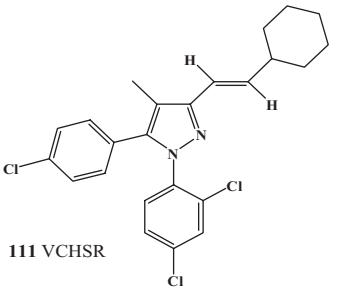
108 BML-190



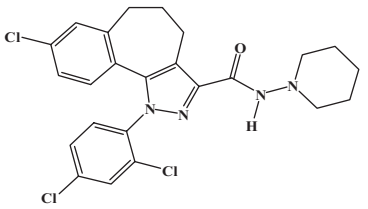
109 L-768242



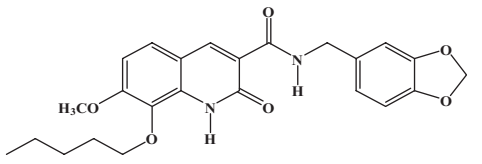
110 L-759787



111 VCHSR

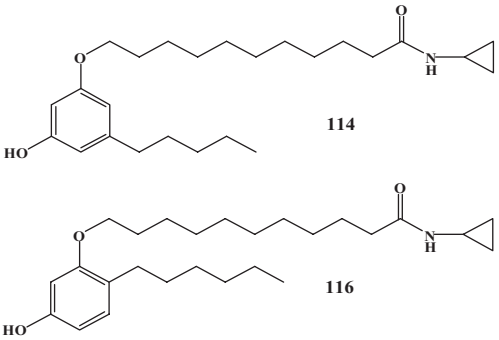


112 NESS 0327

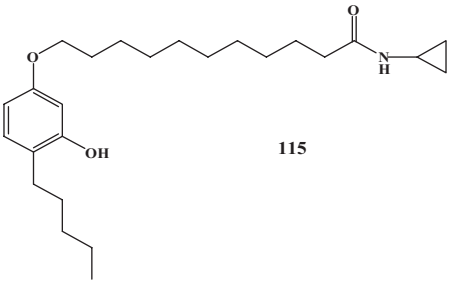


113 JTE907

New potent compounds with high affinity to CB_1 and CB_2 receptors were designed, synthetized and biologically evaluated by an Italian scientific group.[302] The compounds **114** showed nanomolar affinity for both receptors. The highest selectivity for the CB_2 receptor were shown by compound **115** (a 26-fold selectivity over CB_1 receptors—350 nM and >10 μM) and **116** (30 and 210 nM). These cyclopropylamides are more potent than the respective ethanolamides.



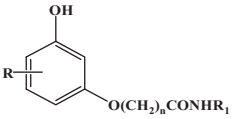
114



115

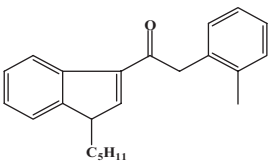
116

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, $N = 7$, $R_1 = c$-C_3H_5) revealed for the CB_1 receptor, activity of $K_i = 56$ nM and for the CB_2 receptor, 280 nM.

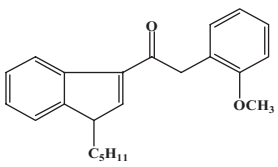


117

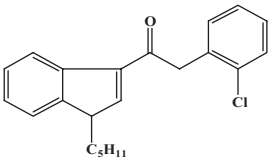
A new class of cannabimimetic indoles, 1-pentyl-3-phenylacetylindoles, has been prepared and their affinity to the cannabinoid receptors studied.[304] Compounds JWH-251 (**118**) and JWH-302 (**119**) were found moderately selective for the CB_1 receptor (29 and 17 nM) and are full agonists at this receptor and partial agonists at the CB_2 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).



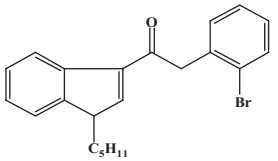
118 JWH-251



119 JWH-302



120 JWH-203



121 JWH-249

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).

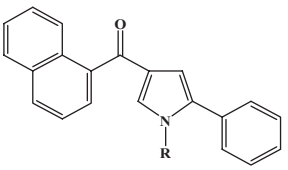
Carpino et al.[306] prepared and evaluated a series of conformationally-constrained bicyclic, selective central cannabinoid receptor CB_1 antagonists and inverse agonists. Two of these compounds (**124** and **125**) showed high selective affinities for central cannabinoid receptors ($K_i = 0.3$ nM and 0.6 nM for hCB_1 and for hCB_2; 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 CB_1 antagonist with moderate affinity to CB_1 receptor. This new neutral cannabinoid CB_1 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 CB_1 cannabinoid ligands.[309] All tested substances displayed only moderate or low CB_1 receptor affinity.

Lavey et al.[310] described triaryl bis-sulfones as a novel class of specific CB_2-receptor ligands. Four of these compounds (**129**–**132**) with high affinity for the human receptor are such CB_2-selective ligands. Study of the most potent and selective compound, Sch225336 (**131**) (K_i for CB_2 0.4 nM and for CB_1 905 nM, respectively), suggest that the class are inverse agonists for the CB_2 receptor.

CB_2-selective radioligand [^{35}S]Sch225336 (**133**) was synthesized and used for detail auto-radiographic analysis of CB_2 in lymphoid tissues.[311]



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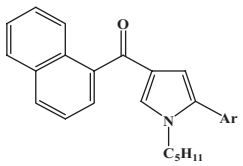

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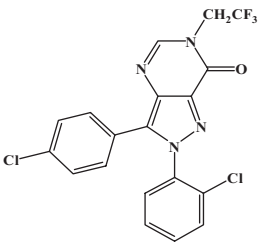

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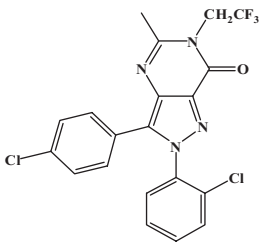

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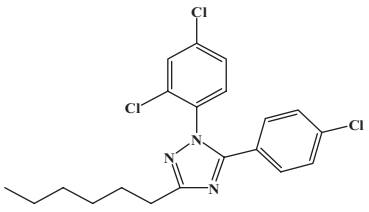
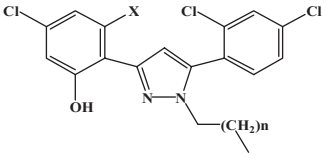


126 LH-21



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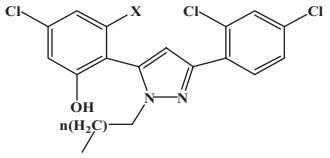

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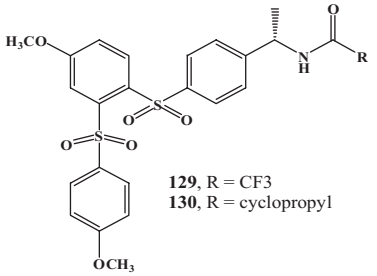
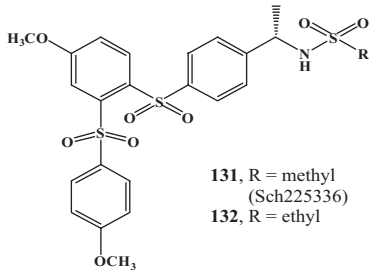
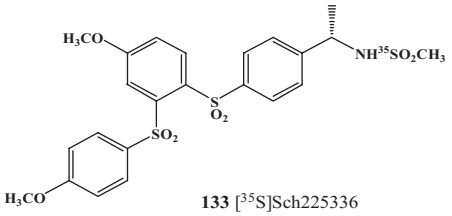


129, R = CF3
130, R = cyclopropyl



131, R = methyl (Sch225336)
132, R = ethyl



133 [^{35}S]Sch225336

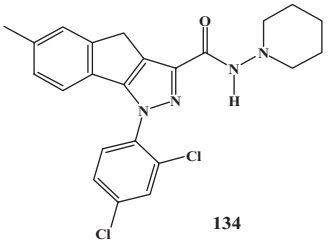
Recently the synthesis and the CB_2-affinity of several new 1,4-dihydroindenol[1,2-*c*]pyrazol-based ligands was reported.[312] Seven of the new compounds displayed very high *in vitro* CB_2 binding affinities (K_i between 0.34 and 0.9 nM). Compounds **134** and **135** showed the highest selectivity for the CB_2 receptor while compound **136** had the highest affinity *and* selectivity. Another series of analogues of the previously reported CB_2 ligands revealed several compounds with CB_1 affinity in nM range with moderate or negligible affinity towards CB_2 receptors.[313] This scientific team prepared a series of new ligands, tricyclic pyrazoles, with high CB_2 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 CB_2 selectivity (K_i = 37 pM, 340 pM, 7.6 nM, and 32.8 nM) against CB_1 affinity (K_i = 363 nM, 2.05 μM, 900 nM, and 2,409 μM).

Recently the identification of novel, selective, and potent CB_2 receptor agonists was reported.[315] A novel series of sulfonamide derivatives (**138**) was synthetized and evaluated. The presence of a 1-Naphthyl group (**139**) resulted in a significant increase in the affinity for the CB_2 receptor (CB_2 IC_{50} = 16 nM, CB_1 IC_{50} = 1.7 μM). This compound was found to be a full CB_2 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 CB_1 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 CB_1 binding affinity.[317] Four of these compounds (**141**–**144**) were able to cause at least 50% displacement of radioligand from CB_1 receptors (K_i for these four particular compounds found 42, 37.5, 39, and 20 μM).

Recently, Horswill et al.[318] provided evidence for allosteric antagonism of the CB_1 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.



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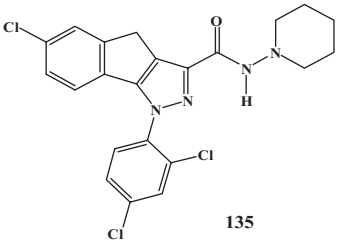

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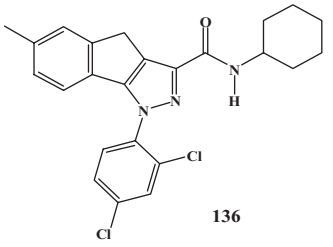

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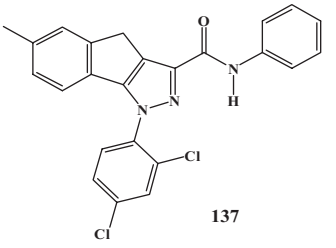

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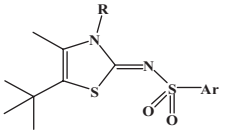

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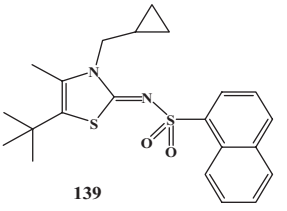

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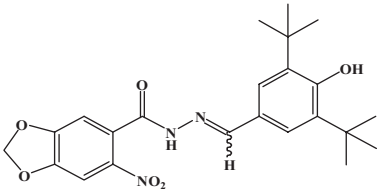


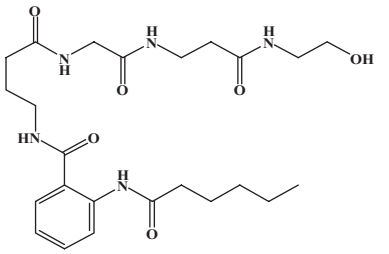
140 LASSBio-881

A CB_1 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 [^{11}C]MePPEP (**146**) with high affinity and selectivity for CB_1 receptor ($K_b = 0.574 \pm 0.207$ nM) compared to $CB_2 = 363 \pm 87.4$ nM, which is a CB_1 mixed inverse agonist and antagonist, was used to image CB_1 receptors in monkey brain. The uptake of [^{11}C]MePPEP in nonhuman primate brain demonstrated reversible labeling of CB_1 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.



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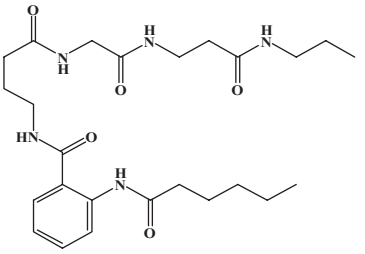

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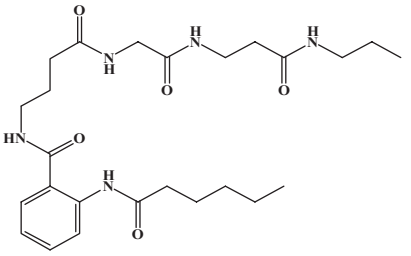

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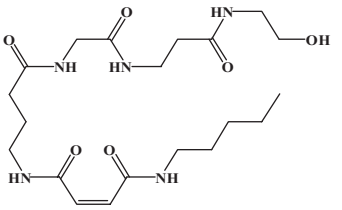

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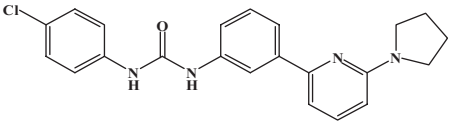
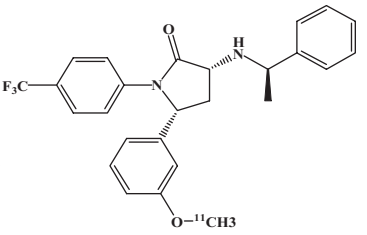


145 PSNCBAM-1



146 [^{11}C]MePPEP

The therapeutic importance of the endocannabinoid system is today without any doubt well recognized. This subject has appeared recently in books[330] 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 CB_1 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 CB_1 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 CB_1 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 CB_1 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 CB_1 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 CB_1 receptor antagonists, should be considered as useful adjuncts to lifestyle and behavior modifications in the treatment of obesity.[356]

Cannabinoid CB_1 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 CB_1 and/or CB_2 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 CB_1 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 CB_1 receptors reduces the neuroendocrine response and does not affect the behavioral response to moderate stress. Finally, the CB_1 receptor plays a role in modulating the stress response from an early age. These observations suggest that CB_1 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 CB_1 and CB_2 receptor subtypes. Guindon and Hohmann[373] reviewed behavioral, neurochemical and electrophysiological data, which identify cannabinoid CB_2 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 CB_2-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 CB_1 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 Costa[376] 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 Hitchcock[377] reviewed the present development of cannabinoid agonists with an emphasis on selective CB_2 agonists and peripherally restricted CB_1 or CB_1/CB_2 dual agonists for treatment of inflammatory and neuropathic pain.

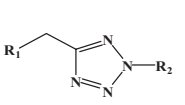
Guy et al.[378] invented the use of a combination of cannabidiol and Δ^9-tetrahydrocannabinol in the treatment of neuropathic pain, in particular peripheral neuropathic pain.

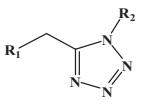
The effects of the synthetic cannabinoid WIN 55,212-2 were examined in a rodent model for neuropathic pain. Their results provide a neural basis for reports of potent suppression by cannabinoids of the abnormal sensory responses that result from nerve injury.[379]

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** (IC_{50} 2.3 μM), **148** (IC_{50} 5.1 μM), **149** (IC_{50} 4.9 μM), **150** (IC_{50} 5.0 μM), and **151** (IC_{50} 5.1 μM), inhibit anandamide cellular uptake process with a relative high potency. Such compounds could contribute to potential therapy of different diseases.





147, R_1 = 4-biphenyl-4-carboxamido; $R_2 = CH_2CON(CH_3)_2$
148, R_1 = 4-biphenyl; $R_2 = CH_2CN$
149, R_1 = 4-biphenyl; $R_2 = CH_2CO_2CH_3$
150, R_1 = 4-biphenyl; $R_2 = CH_2COCH_3$
151, R_1 = 4-biphenyl; $R_2 = CH_2CON(CH_3)_2$

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.

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