

REVIEW**Discovery and Isolation of Anandamide and Other Endocannabinoids**by **Lumír O. Hanuš**Department of Medicinal Chemistry and Natural Products, School of Pharmacy, 12065,
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(phone: +972-2-6758042; e-mail: lumir@cc.huji.ac.il)**Contents**

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1. Introduction. – Cannabis had many uses as a cultivated plant already in Neolithic China [1]. The first known record of the use of cannabis as a medicine was published in China 5000 years ago in the reign of the Emperor *Chen Nung*. However, it was not until the 19th century that cannabis became a common medicine in Europe, and its analgesic, anticonvulsant, anti-spasmodic, anti-emetic, and hypnotic uses were recognized.

The Assyrians, who ruled large parts of the Middle East for nearly a millennium, *ca.* 3000 years ago, have left us a pharmaceutical legacy on hundreds of clay tablets. Cannabis was one of the major drugs of their pharmacopoeia. They named this plant according to its use. *Campbell* [2] identified the Sumerian term *a-zal-la* and the Akkadian term *azulla* as cannabis on the basis of their similarities to the Syrian *azal*, meaning ‘to spin’. He also took the Assyrian word *gurgurangu* 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 *gan-zi-gun-nu* as hashish (a robber (*gan*) who spins away (*gun-nu*) the soul (*zi*)), and this word has been translated as ‘*the drug that takes away the mind*’ (from today point of view we can translate it as ‘*cannabimimetic*’).

A letter written around 680 B.C. by an unknown woman to the mother of the Assyrian king, *Esarhaddon*, mentions a substance called *qu-nu-bu* which also may have been cannabis, but again there is no certainty for this identification [3].

Pliny the Elder, an ancient Roman nobleman, scientist, and historian, author of *Naturalis Historia*, ‘*Pliny’s Natural History*’ (79 A.D.) wrote that ‘*The roots boiled in water ease cramped joints, gout too and similar violent pain*’ [4].

Pedanius Dioscorides (ca. 40 in Anazarbus, Cilicia – ca. 90), an ancient Greek physician, pharmacologist, and botanist who practiced in Rome at the times of Nero (90 A.D.) mentions that ‘*The sodden root when placed on inflammations soothes them, eliminates edema and disperses obdurate matter above inflamed joints*’ [5].

French physician *Jacques Joseph Moreau* remains 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 he also recruited other members of France’s artistic community (*Charles Pierre Baudelaire*, one of the greatest poets of the 19th century; *Honoré de Balzac*, *Alexandre Dumas*, and *Gustave Flaubert*). This crew of experimenters donned around 1835 the name the *Club des Hashichins* (*Hashish Club*), and met monthly in an old mansion in Paris [6].

The single most complete and authoritative work on the history of the genus *Cannabis* published *Abel* [7].

2. Cannabinoids in Plant and Body. – In the plant *Cannabis sativa* L. and its phytochemical products (hashish, marihuana), the total number of identified compounds of many different types is today 489. Seventy of them, known as cannabinoids, are the typical C₂₁ group of compounds which are present in plant kingdom only in this plant [8].

The whole story about the isolation of cannabinoids from the plant was published in detail in [9]. Briefly, the main cannabinoids are cannabidiol, Δ^9 -tetrahydrocannabinol, and cannabinol. The first successful attempt to identify the first of *Cannabis*-typical compounds (cannabinoids), cannabinol, was achieved by *Wood et al.* [10][11]. *Cahn* almost elucidated the structure of cannabinol [12]. The correct structure of this compound was solved by *Todd* and co-workers [13][14] and independently by *Adam et al.* [15].

When a second cannabinoid, cannabidiol, was isolated, its structure was clarified only partially [16]. Synthetic tetrahydrocannabinol derivatives, which showed cannabis-like activity in animal tests, were prepared, but they obviously differed from the active natural product [17–20]. *Krejčí* and *Šantavý* isolated cannabidiolic acid and reported a nearly correct structure [21][22].

In 1963, *Mechoulam* and *Shvo* isolated cannabidiol, and reported its correct structure and configuration [23]. A year later, *Gaoni* and *Mechoulam* finally succeeded in isolating pure (–)-*trans*- Δ^9 -tetrahydrocannabinol (Δ^9 -THC) and elucidated its structure [24]. The absolute configuration of cannabidiol and of THC was established by a shift of the optical-rotation value [25] and by correlation with known terpenoids [26]. Several years later, a minor psychotomimetically active constituent, Δ^8 -THC, was isolated from marijuana [27].

Several additional cannabinoids were also identified at that time. The best known are cannabigerol [28], cannabichromene [29][30], and cannabicyclol [31] (*Fig. 1*).

The active ingredient of cannabis is (–)-*trans*- Δ^9 -tetrahydrocannabinol (Δ^9 -THC), and it is thought to exert its effect by binding to cannabinoid CB₁ receptors on pre-synaptic nerve terminals in the brain. Δ^9 -THC binding to CB₁ receptors activates G

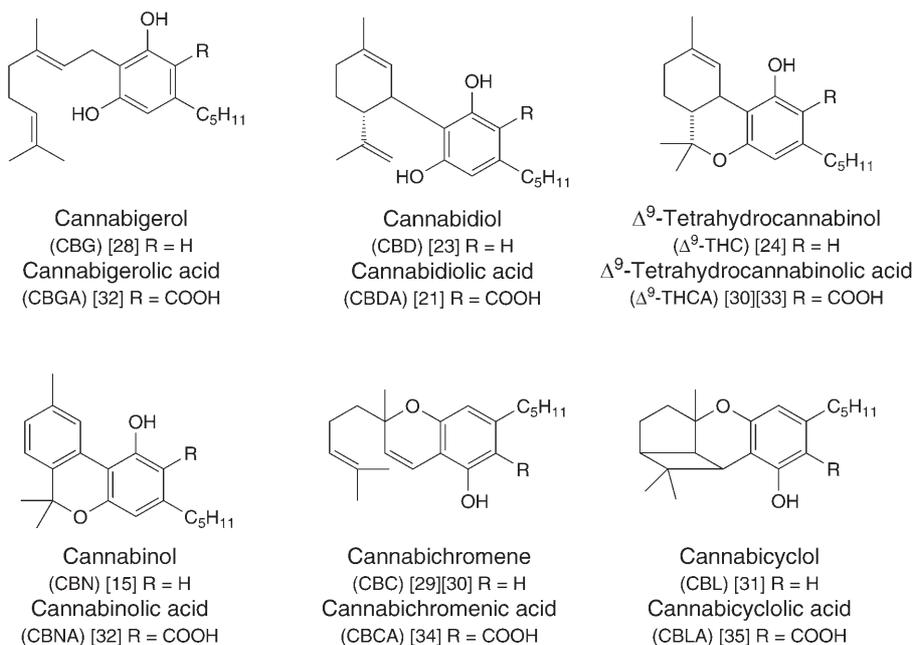


Fig. 1. Representative natural cannabinoids isolated from *Cannabis sativa L.*

proteins that activate/inhibit a number of signal transduction pathways. The G proteins directly inhibit N- and P/Q-type voltage-dependent calcium channels and sodium channels, and indirectly inhibit A-type calcium channels *via* inhibition of adenylate cyclase. Δ^9 -THC Binding and G-protein activation also activates inwardly rectifying potassium channels and the MAP kinase signalling pathway. The cumulative effect of these pathways is the euphoric feelings associated with cannabis use.

Since CB_1 receptors are not found in the part of the brain which is responsible for respiratory and cardiovascular functions, using a compound having an affinity to CB_1 receptors (namely a CB_1 -binding cannabinoid) is not associated with an increased risk of respiratory or cardiovascular failure, which is common with many other drugs, and is, therefore, advantageous therapeutic agents [36].

The cannabinoid activity is strongly dependent on stereochemical factors. $\Delta^{6a,10a}$ -THC in its racemic form was synthesized in early 1940s [18][37]. Although attempts were made to obtain the enantiomers in a pure form, these were only partially successful. After *Mechoulam* and co-workers able to synthesize (1*S*)- Δ^3 -THC and (1*R*)- Δ^3 -THC with absolute optical purity, these enantiomers have been tested in human volunteers [38]. Thus, it was demonstrated, for the first time, in man that, within a pair of cannabinoid enantiomers, only the (1*S*)-enantiomer is cannabimimetic (had definite psychic actions).

The natural Δ^9 -THC and Δ^8 -THC are (3*R*,4*R*)-isomers. The synthetic route which was developed nearly 20 years ago makes also possible the synthesis of the (3*S*,4*S*)-enantiomers [39].

With the synthesis of absolute pure (–)-7-OH- Δ^8 -THC-DMH (HU-210) and (+)-7-OH- Δ^8 -THC-DMH (HU-211), the (+)-enantiomer was found to be inactive [40]. So, cannabimimetic activity in man has strict stereochemical requirements, which indicated a probable interaction with a chiral biological system (enzyme, receptor site *etc.*). The results with enantiomeric compounds clearly show that, in the enantiomeric pair of THC-type compounds (HU-210 and HU-211), psychotropic activity resides solely in the (–)-(3*R*,4*R*)-enantiomer, the (+)-(3*S*,4*S*)-enantiomer being inactive at doses up of several thousand times higher than the (–)-(3*R*,4*R*)-enantiomer [41–43]. So, natural compound, (–)- Δ^9 -THC (equatorial enantiomer), is the active one, and its synthetic enantiomer, (+)- Δ^9 -THC (axial enantiomer), is inactive.

3. Cannabinoid Receptors. – Δ^9 -THC causes its effects by interacting with specific cannabinoid receptors CB₁ and CB₂ [44][45]. CB₁ is localized mainly in the brain and testis, and CB₂ is localized in peripheral tissues such as the spleen, tonsils, and immune cells. The CB₁ receptor is a G protein-coupled receptor. The CB₂ receptor has structural features similar to those of the CB₁ receptor, but it is of a smaller size.

There is a widespread distribution of CB₁ receptors throughout the brain, in regions whose functionality correlates well with the known effects of cannabinoids [36]. There is high CB₁-receptor density in the basal ganglia, hippocampus, and cerebellum. The hippocampus is a brain region which is essential for the storage of newly acquired information. This may explain the involvement of cannabinoids in the impairment of cognition and memory. The basal ganglia is a brain region associated with locomotor activity. CB₁ Expression in this region helps to explain cannabinoid-induced effects such as involuntary movements and loss of motor control. The cerebellum is associated with distal limb coordination, posture, and balance. The presence of a high density of CB₁ receptors is consistent with the effect of cannabinoids on motor function and motor learning. The presence of CB₁ receptors in the testis may be responsible for cannabinoid-induced effects on the reproductive system such as decreased sperm count.

The CB₂ receptor is found in immune tissues such as spleen, tonsils, and immune cells [46]. The presence of the CB₂ receptor in immune tissues suggests that cannabinoid-induced immunosuppression may be a CB₂ receptor-mediated process.

Signal transduction is the mechanism by which intracellular processes recognize the activation of a receptor on the outer surface of the cell. When activated, CB₁ and CB₂ receptors couple to signal transduction pathways including adenylate cyclase, mitogen-activated protein (MAP) kinase, and ion channels [47].

The CB₁ receptor modulates the activity of voltage-dependent Ca²⁺ channels and enhances the activation of voltage-dependent K⁺ channels, and inwardly rectifying K⁺ channels. Activation of the CB₁ receptor stimulates the coupling to a G protein. The G protein stimulates MAP kinase and inhibits adenylate cyclase, which leads to decreased production of cyclic adenosinemonophosphate (cAMP). The G protein directly couples the CB₁ receptor to voltage-dependent Ca²⁺ channels and stimulates inwardly rectifying K⁺ channels. The decrease in cAMP accumulation inhibits the cAMP-dependent production of protein kinase A (PKA). The phosphorylation of the A-type K⁺ channel by cAMP-dependent PKA is reduced.

Due to the low homology of the two receptors, the CB₂ receptor transduces different signals compared to the CB₁ receptor. Like the CB₁ receptor, the CB₂ receptor

couples to signal transduction pathways inhibiting adenylate cyclase and stimulating MAP kinase, but it has not been shown to modulate ion channels.

As well as Δ^9 -THC, the active ingredient of the plant *Cannabis sativa*, a number of other compounds also activate the cannabinoid receptors. Cannabinoid receptor agonists can be subdivided into four groups according to their chemical structure. They all have pharmacological and behavioral effects similar to Δ^9 -THC. These are classical cannabinoids, *i.e.*, plant-derived cannabinoids, eicosanoid cannabinoids, animal-derived cannabinoids, non-classical cannabinoids, *i.e.*, synthetic cannabinoids and amino-alkylindoles, and synthetic cannabinoids [48–50].

Ca^{2+} Currents are required for neurotransmitter release at CNS synapses. Cannabinoids activate the CB_1 receptor, which inhibits pre-synaptic Ca^{2+} channels, which is the likely mechanism for the inhibition of neurotransmitter release from CB_1 expressing pre-synaptic terminals.

The inhibition of Ca^{2+} channels may be the mechanism by which cannabinoids inhibit acetylcholine release in the hippocampus, inhibit noradrenaline release at sympathetic nerve terminals and in the hippocampus, cortex, and cerebellum.

In leukocytes, the cannabinoid-induced inhibition of adenylate cyclase, leading to a reduction in cAMP signaling, is correlated with decreased immune function.

Mitogen-activated protein kinase (MAPK) activation may be an intermediate step in the induction of transcription factor, *krox 24*, which is involved in cell differentiation and development.

Both Δ^9 -tetrahydrocannabinol, the psychoactive component of *Cannabis sativa*, and anandamide, an endogenous neurotransmitter in our brain, bind to the same cannabinoid receptor, which shows high levels of expression in the brain. Binding of Δ^9 -tetrahydrocannabinol and anandamide to the cannabinoid receptor reduces the excitability of the respective neuron and, thus, interneuronal communication.

4. Isolation of Anandamide. – After *Pert* and *Snyder* [50][51], first identified opioid receptors in the brain in 1972, in 1975 *Kosterlitz* and *Hughes* [52] reported the existence of an endogenous morphine-like substance, opioid neuropeptide. Later, they named it endorphin (endogenous morphine) enkephalin. Abilities of this endogenous opioid biochemical compounds to produce analgesia and a sense of well-being designated them as ‘natural pain killers’. A similar role, as was found later, play endocannabinoids.

The discovery of a high-affinity, stereoselective, and pharmacologically distinct cannabinoid receptor in a rat brain tissue [44] led us to a search for natural endogenous ligands in the brain, which bind to and activate this cannabinoid receptor. The existence of cannabinoid receptors suggested the presence of endogenous ligands. It was unmistakable, without any doubt, that the cannabinoid receptor is not present in the brain because of some psychotomimetically active plant constituents from marijuana and hashish, but to be activated by specific endogenous ligands. The synthesis of a specific, highly potent radiolabelled probe (agonist) [^3H]HU-243, which binds to the CB_1 receptor, made possible a sensitive bioassay [53]. HU-243 was actually found to be the most active cannabinoid known so far. In a standard bioassay, we expected that endogenous compounds with cannabinoid activity would displace tritiated HU-243 bound to the central cannabinoid receptor (CB_1). Porcine brain fractions were found to compete with this probe for binding to cannabinoid receptors. Chromatography of such

brain fractions led to the identification of a family of unsaturated fatty acid ethanolamides with THC-like activity [54][55].

Our search for an endogenous ligand for the cannabinoid receptor in the brain led us to the tedious and long-lasting isolation of an unknown compound. Our effort was crowned by success on March 24, 1992. This day, we had in our hands hitherto an unidentified active ligand for the central cannabinoid receptor. That day, the identification of this compound started. Next day, we established, with the help of bioassay, that this compound is active in binding on the cannabinoid receptor. Thin-layer chromatography showed just one compound, gas chromatography gave us one tailing peak, and, after silylation, a nice peak. On May 13, 2002, we recorded the mass spectrum (M_r 329; Fig. 2), and, the next day, the mass spectrum of this compound after silylation (M_r 419, implying that M_r is 347; Fig. 3). There was a discrepancy between the mass spectra of silylated and non-silylated compounds. The difference of 18 indicated that the non-silylated sample underwent dehydration during analysis. The structure elucidation of this compound started, and, on July 13, the structure was solved and later confirmed by the synthesis and spectroscopic comparison.

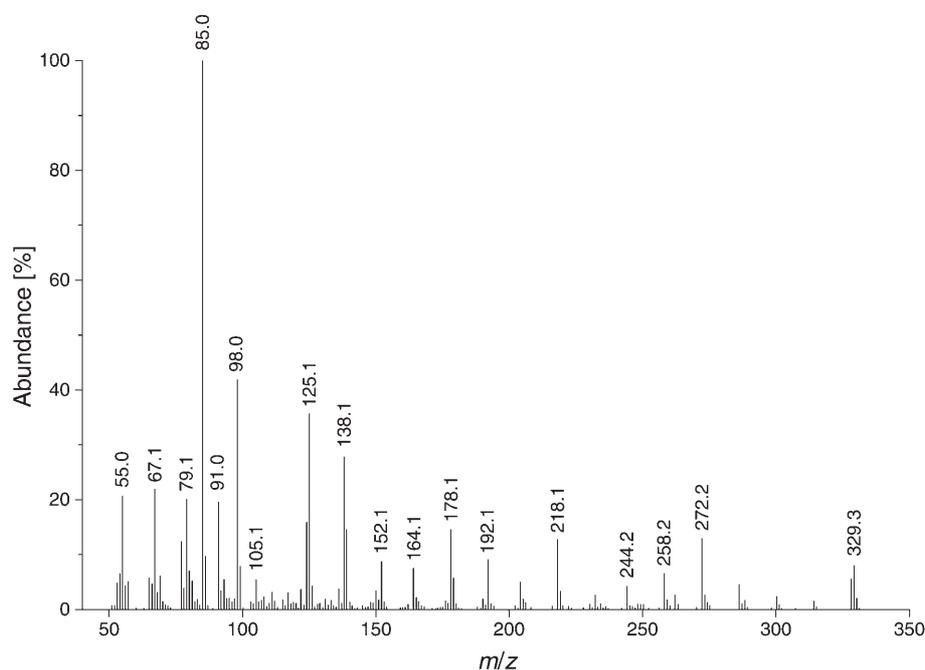


Fig. 2. Original mass spectrum of anandamide

The structure of anandamide (Fig. 4) was established by mass spectrometry and NMR spectroscopy. A variety of MS measurements were performed on the purified material (GC/MS analyses, high-resolution MS measurements, collision-induced dissociation measurements). Direct-exposure chemical ionization (isobutene-DCI) MS indicated a molecular weight of 347 (m/z 348 ($[M + H]^+$)). High-resolution MS measurement suggested the elemental composition of $C_{22}H_{37}NO_2$ (m/z 347.2762),

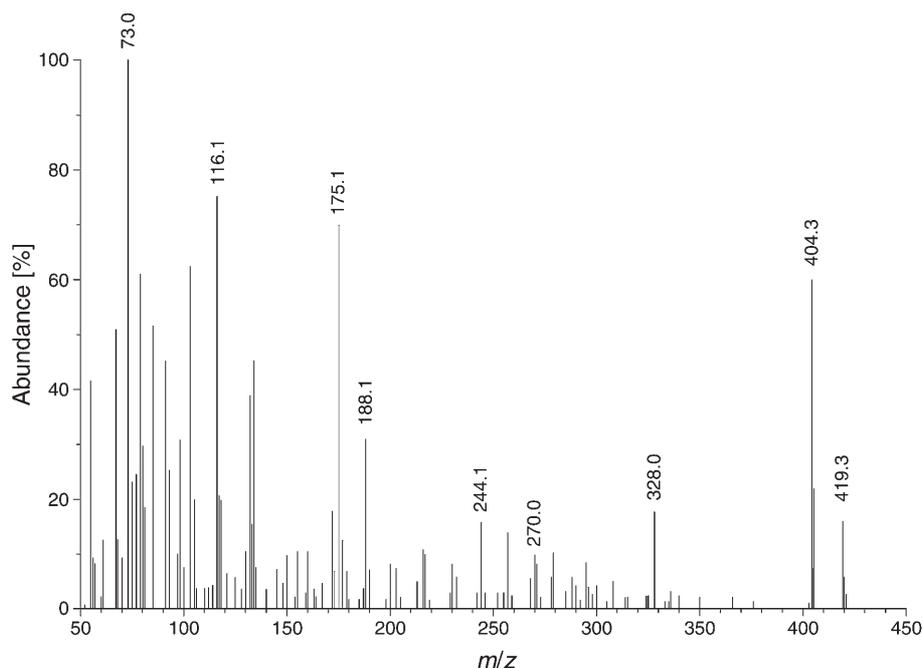


Fig. 3. Original mass spectrum of the trimethylsilyl derivative of anandamide

which is consistent with the presence of five C=C bonds or rings. Collision-induced dissociation (CID) measurement of the m/z 348 $[M+H]^+$ ion obtained under isobutene-DCI gave rise to the following significant fragments: m/z 287, 245, 203, 62 (highest abundance), and 44. The only reasonable composition of the most abundant m/z 62 fragment ion is C_2H_8NO , which corresponds to the protonated ethanolamine $HOCH_2CH_2NH_3^+$. The m/z 44 ion may be formed by dehydration of the m/z 62 fragment. The m/z 287 ($[M+H-61]^+$) fragment ion corresponds to the loss of ethanolamine (C_2H_7NO) from $[M+H]^+$. Additional data were obtained from the GC/MS and CID measurements of the Me_3Si (TMS) derivative of the purified material. Together, these results suggested that anandamide is an ethanolamide of arachidonic acid.

Supporting evidence for this general structure was found in the behavior of anandamide under GC/MS conditions. Thermal dehydration gave rise to the M^+ ion at m/z 329 upon electron ionization (EI) and to $[M+H]^+$ ion under CI. Both self-CI m/z 330 ($[M+H]^+$) and m/z 329 (M^+) were formed under EI conditions in an ion-trap instrument. The fragmentation pattern of dehydration products of anandamide and palmitoylethanolamide were similar in the low-mass range of the EI mass spectra, and included m/z 85 (*McLafferty* rearrangement ion) and m/z 98 (product of a γ -cleavage). The EI mass spectrum of dehydrated palmitoylethanolamide exhibited an ion at m/z 112 that corresponded to a δ -cleavage fragment. The absence of this ion in the EI mass spectrum obtained from the GC/MS analysis of anandamide the presence of the first

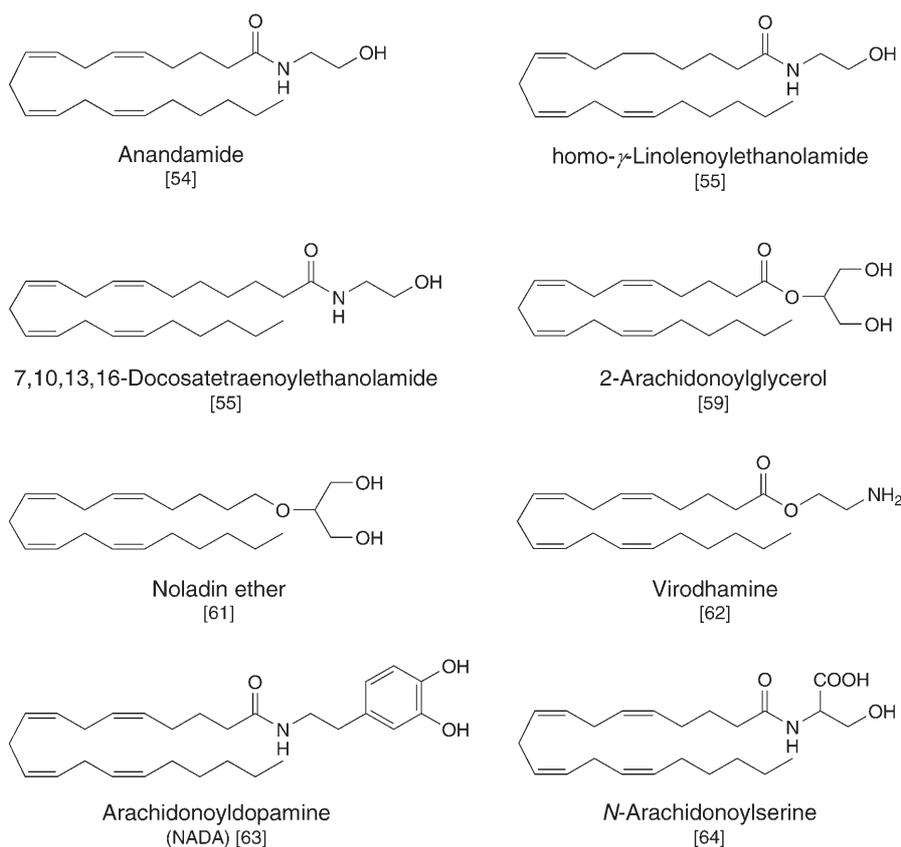


Fig. 4. Up-to-date known endocannabinoids

C=C bond in the tetraenic acid at position 5 (as in arachidonylethanolamide, which would not be expected to yield a δ -cleavage product).

Because of the small amount of natural anandamide available, we were able to record only $^1\text{H-NMR}$ spectra. The peaks attributed to $\text{CH}=\text{CH}$ H-atoms (δ 5.30–5.45, *multiplet*) were coupled with those of the H-atoms that have the chemical shifts of doubly allylic H-atoms (δ 2.75–2.90, *multiplet*). Such doubly allylic H-atoms are typically found in all-*cis*, nonconjugated polyunsaturated fatty acids as linoleic and arachidonic acids. Three pairs of H-atoms were observed between δ 2.01 and 2.27, which we attributed to two allylic CH_2 groups and one CH_2 group in α -position to a C=O moiety. Only one Me group was observed (0.99, *triplet*). The peaks observed for two H-atoms at 3.42 (CH_2N , *triplet*), two H-atoms at 3.72 (CH_2O , *triplet*), and two H-atoms at 2.20 (COCH_2 , *triplet*) were similar in chemical shifts and spin-coupling patterns to peaks observed in the NMR spectrum of synthetic palmitoylethanolamide. The peaks for CH_2N and CH_2O were coupled.

A juxtaposition of the various analytical data led us to conclude that the structure of anandamide is that of arachidonylethanolamide. This conclusion was confirmed by synthesis.

Anandamide inhibited the specific binding of [³H]-HU-243 to synaptosomal membranes in a manner typical of competitive ligands, with an inhibition constant (K_i) of 39.0 ± 5.0 nM. In this system, the K_i value of Δ^9 -THC, the psychoactive compound of *Cannabis sativa*, was 46.0 ± 3.0 nM. Exciting results: a compound from a higher plant and a chemically different compound from the brain bind to the same receptor in the brain with about the same activity.

We named the active constituent anandamide, based on the Sanskrit word *ananda* meaning delight, bliss, and on its chemical nature. At the same time, this name expressed the feelings of the discoverers after this success [52]. Soon after this discovery, this compound was tested for its pharmacological activity [56].

Anandamide is either a neuromodulator or neurotransmitter. This recently discovered messenger molecule plays a role in pain, depression, appetite, memory, and fertility. Anandamide and 2-arachidonylethanolamide (2-AG) are endogenous cannabinoids that were shown to be protective using *in vitro* models of ischemia [57]. Mounting *in vitro* and *in vivo* data suggest that the endocannabinoids anandamide and 2-arachidonylethanolamide, as well as some plant and synthetic cannabinoids, have neuroprotective effects following brain injury [58]. Cannabinoid receptor agonists inhibit glutamatergic synaptic transmission and reduce the production of tumor necrosis factor- α and reactive oxygen intermediates, which are factors in causing neuronal damage. The formation of the endocannabinoids anandamide and 2-arachidonylethanolamide is strongly enhanced after brain injury, and there is evidence that these compounds reduce the secondary damage incurred. Some plant and synthetic cannabinoids, which do not bind to the cannabinoid receptors, have also been shown to be neuroprotective, possibly through their direct effect on the excitatory glutamate system and/or as antioxidants.

5. Other Endocannabinoids. – Our research group expected that additional polyunsaturated fatty acid ethanolamides may be present in the brain, and identified in porcine brain another two putative endocannabinoids, namely homo- γ -linoleylethanolamide (*cf. Fig. 4*; $K_i = 53.4 \pm 5.5$ nM) and 7,10,13,16-docosatetraenylethanolamide ($K_i = 34.4 \pm 3.2$ nM). Isolation of these two compounds [55] as constituents of porcine brain that bind to the cannabinoid receptor demonstrated that anandamide is not the sole representative of this class of potential mediators.

Later, we described the isolation of a second type of cannabinoid receptor ligand, 2-arachidonylethanolamide (2-AG; $K_i = 5.85 \pm 0.12$ μ M), an ester isolated from canine gut [59]. This was the first putative endogenous cannabinoid receptor ligand isolated from a peripheral tissue. Later, *Sugiura et al.* isolated independently this compound from brain [60].

2-Arachidonylethanolamide glyceryl ether (noladin ether), isolated from porcine brain, is an example of a third, ether-type endocannabinoid [61]. The name is derived from the Hebrew word *nolad*, which means ‘to be born’. The structure of noladin ether was determined by mass spectrometry and NMR spectroscopy, and was confirmed by comparison with a synthetic sample. It binds to the CB₁ cannabinoid receptor ($K_i =$

21.2 ± 0.5 nM) and causes sedation, hypothermia, intestinal immobility, and mild antinociception in mice. It binds weakly to the CB₂ receptor ($K_i > 3 \mu\text{M}$).

A compound with the same molecular weight as anandamide, but with a shorter retention time, was identified as *O*-arachidonylethanolamine (arachidonic acid and ethanolamine connected by an ester linkage) ($EC_{50} = 1906 \text{ nM}$). Based on this opposite orientation, the molecule was named virodhamine from the Sanskrit word *virodha*, which means ‘opposition’ [62].

Huang *et al.* [63] assumed that *N*-arachidonoyldopamine (NADA) may exist as an endogenous ‘capsaicin-like’ cannabinoid in mammalian nervous tissues and may possibly bind to the vanilloid receptor VR1. They found that NADA is indeed a natural endocannabinoid, in nervous tissues, with high concentrations found in the striatum, hippocampus, and cerebellum, and with lower concentrations, in the dorsal root ganglion. NADA binds to the cannabinoid receptors with a 40-fold selectivity for the CB₁ ($K_i = 250 \pm 130 \text{ nM}$) over the CB₂ receptors.

It seemed reasonable to expect that the chemically closely related *N*-arachidonoyl-L-serine (ARA-S) could also be formed alongside anandamide (*cf.* Fig. 4). This compound was isolated from bovine brain, and its structure was elucidated by comparison with synthetic ARA-S [64]. Contrary to anandamide, ARA-S binds very weakly to the known cannabinoid CB₁ and CB₂, or vanilloid TRPV1 receptors. However, 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. 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 in CB₁ or CB₂ receptors. Many of these effects parallel those reported for abnormal-cannabidiol (Abn-CBD), a synthetic agonist of a putative novel cannabinoid-type receptor [65][66]. Hence ARA-S may represent an endogenous agonist for this receptor.

6. The Endocannabinoid Congeners. – The endocannabinoids are accompanied by cannabinoid receptor-inactive, saturated, and mono- or diunsaturated congeners which can influence their metabolism and function (Fig. 5).

Palmitoylethanolamide, which was isolated from rat and quinea pig brains [67], has been shown to exhibit antiinflammatory and analgesic activity even though it does not activate central and peripheral cannabinoid receptors [68][69]. Palmitoylethanolamide binds to the orphan G protein-coupled receptor GPR55 (which might represent a new cannabinoid receptor) and is potent stimulant ($EC_{50} = 3.2 \pm 1.3 \text{ nM}$) [70].

It was found that, in spleen, brain, and gut, 2-AG is accompanied by several 2-acylglycerol esters, two major ones being 2-linoleoylglycerol and 2-palmitoylglycerol, which significantly potentiate the apparent binding of 2-AG and its apparent capacity to inhibit adenylyl cyclase. The data indicated that the biological activity of 2-AG can be increased by related, endogenous 2-acylglycerols, which alone show no significant activity in any of the tests employed. This ‘entourage effect’ may represent a novel route for molecular regulation of endogenous cannabinoid activity [71].

Another saturated ethanolamide, stearoylethanolamide, exerts a marked dose-dependent anorexic effect. This congener reduces food intake in mice in a structurally selective manner [72]. Maccarone *et al.* [73] reported that stearoylethanolamide binds

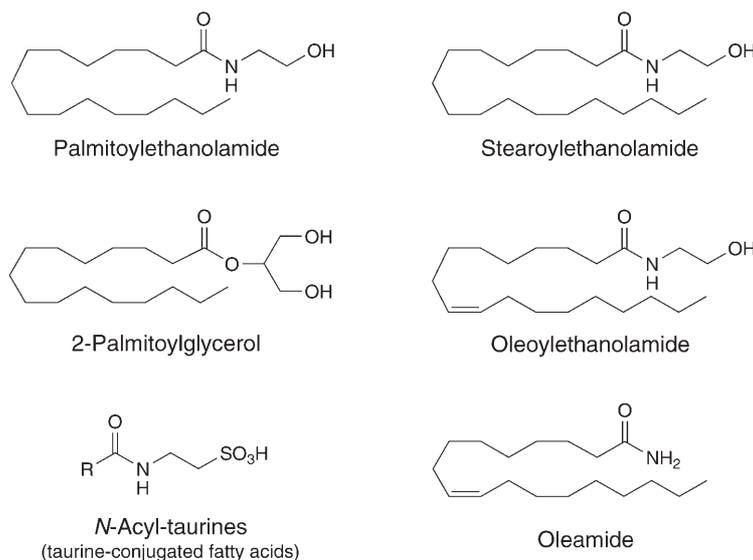


Fig. 5. Some endocannabinoid congeners

to a specific site different from known cannabinoid or vanilloid receptors, is not coupled to G proteins, and regulates different signaling pathways. Degradation and pro-apoptotic activity of stearoylethanolamide are 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 SEA might also be an 'entourage' compound [74].

Oleoylethanolamide is an endogenous regulator of food intake, and intraperitoneal injection of this compound decreased food intake in 24-h-starved rats [75]. 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 [76]. A possible protective action of oleoylethanolamide against reactive oxygen species could explain its beneficial effects on *in vitro* capacitated spermatozoa [77].

Oleamide, an unsaturated fatty acid amide which can modulate central nervous system function was isolated from the cerebrospinal fluid of sleep-deprived cats [78]. Nanogram amounts of oleamide in biological fluids measured using GC/MS, as a quantitative assay, would aid in determining the role of oleamide in physiological processes [79]. Its hypnotic properties were characterized [80]. Oleamide, a sleep-inducing factor, is the lipid whose mechanism of action is far from being understood. Although it does not bind with high affinity to CB₁ or CB₂ receptors, it exhibits some cannabimimetic actions which could be explained at least in part by entourage effects. It is likely that oleamide and anandamide have common as well as distinct pathways of action. The 5-HT_{2A} receptor appears to be a target for oleamide, but the possibility of the existence of specific receptors for this compound is open [81]. Oleamide is a full

cannabinoid CB₁ receptor agonist. Therefore, in addition to allosteric modulation of other receptors and possible entourage effects due to fatty acid amide hydrolase (FAAH) inhibition, the effects of oleamide may be mediated directly *via* the CB₁ receptor [82]. Oleamide-elicited vasorelaxation in rat-isolated small mesenteric arteries. Its responses in the rat-isolated small mesenteric artery are partly dependent on the presence of the endothelium, activation of Ca²⁺-sensitive K⁺ channels and involve capsaicin-sensitive sensory nerves [83].

Several brain lipids 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 [84]. *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* [85].

7. Conclusions. – Since the discovery of anandamide, a brain constituent, which acts as a ligand for the cannabinoid receptor, 2398 publications appeared, which deal with this compound [86]. Analytical methods for the quantification of this compound in the body were developed. Another two anandamide-type compounds were isolated. There are today additional ligands, which are not ethanolamides. Anandamide and 2-arachidonoylglycerol were intensively studied. What is surprising 14 years after the discovery of the first endogenous ligand, anandamide, is that no endocannabinoid has ever been officially administered to a human. In the past, after discovery of similarly important compounds these were in the clinic use within several months or years. With endocannabinoids it is quite different. We do not think that they are in the ‘shadow’ of cannabinoids, but it is necessary, on the one side, that legal conditions to use it are established, and, on the other side, the research today is much more expensive, so such research must be supported by pharmaceutical companies.

REFERENCES

- [1] H.-L. Li, *Econ. Bot.* **1974**, 28, 437.
- [2] R. C. Thompson, ‘A Dictionary of Assyrian Chemistry and Geology’, Clarendon Press, Oxford, 1936.
- [3] L. Waterman, ‘Royal Correspondence of the Assyrian Empire’, University of Michigan Press, Ann Arbor, 1930, letter 368.
- [4] Pliny the Elder, ‘The Natural History’, Taylor and Francis, Red Lion Court, Fleet Street, London, 1855.
- [5] Pedanius Dioscorides, ‘De Materia Medica’, IBIDIS Press, Johannesburg, 2000.
- [6] J. Green, ‘Cannabis’, Thunder’s Mouth Press, New York, 2002.
- [7] E. L. Abel, ‘Marijuana – The First Twelve Thousand Years’, Plenum Press, New York, 1980.
- [8] M. A. ElSohly, D. Slade, *Life Sci.* **2005**, 78, 539.
- [9] L. O. Hanuš, R. Mechoulam, ‘Cannabinoid Chemistry: An Overview’, in ‘Cannabinoids as Therapeutics’, Eds. R. Mechoulam, Birkhäuser, Basel, 2005, p. 23–46.
- [10] T. B. Wood, W. T. N. Spivey, T. H. Easterfield, *J. Chem. Soc.* **1896**, 69, 539.
- [11] T. B. Wood, W. T. N. Spivey, T. H. Easterfield, *J. Chem. Soc.* **1899**, 75, 20.

- [12] R. S. Cahn, *J. Chem. Soc.* **1932**, 1342.
- [13] A. Jacob, A. R. Todd, *J. Chem. Soc.* **1940**, 649.
- [14] R. Ghosh, A. R. Todd, R. S. Wilkinson, *J. Chem. Soc.* **1940**, 1393.
- [15] R. Adams, B. R. Baker, R. B. Wearn, *J. Am. Chem. Soc.* **1940**, 62, 2204.
- [16] R. Adams, H. Wolff, C. K. Cain, J. H. Clark, *J. Am. Chem. Soc.* **1940**, 62, 2215.
- [17] R. Adams, D. C. Pease, C. K. Cain, B. R. Baker, J. H. Clark, H. Wolff, R. B. Wearn, *J. Am. Chem. Soc.* **1940**, 62, 2245.
- [18] R. Adams, B. R. Baker, *J. Am. Chem. Soc.* **1940**, 62, 2405.
- [19] R. Adams, S. Loewe, D. C. Pease, C. K. Cain, R. B. Wearn, B. R. Baker, H. Wolff, *J. Am. Chem. Soc.* **1940**, 62, 2566.
- [20] R. Ghosh, A. R. Todd, S. Wilkinson, *J. Chem. Soc.* **1940**, 1121.
- [21] Z. Krejčí, F. Šantavý, *Acta Univ. Palacki. Olomuc.* **1955**, 6, 59.
- [22] J. Kabelík, Z. Krejčí, F. Šantavý, *Bull. Narc.* **1960**, 12, 5.
- [23] R. Mechoulam, Y. Shvo, *Tetrahedron* **1963**, 19, 2073.
- [24] Y. Gaoni, R. Mechoulam, *J. Am. Chem. Soc.* **1964**, 86, 1646.
- [25] F. Šantavý, *Acta Univ. Palacki. Olomuc., Fac. Med.* **1964**, 35, 5.
- [26] R. Mechoulam, Y. Gaoni, *Tetrahedron Lett.* **1967**, 1109.
- [27] R. L. Hively, W. A. Mosher, F. W. Hoffmann, *J. Am. Chem. Soc.* **1966**, 88, 1832.
- [28] Y. Gaoni, R. Mechoulam, *Proc. Chem. Soc.* **1964**, 82.
- [29] Y. Gaoni, R. Mechoulam, *J. Chem. Soc., Chem. Commun.* **1966**, 20.
- [30] U. Claussen, F. v. Spulak, F. Korte, *Tetrahedron* **1966**, 22, 1477.
- [31] L. Crombie, R. Ponsford, *Tetrahedron Lett.* **1968**, 5771.
- [32] R. Mechoulam, Y. Gaoni, *Tetrahedron* **1965**, 21, 1223.
- [33] T. Yamauchi, Y. Shoyama, H. Aramaki, T. Azuma, I. Nishioka, *Chem. Pharm. Bull.* **1967**, 15, 1075.
- [34] Y. Shoyama, T. Fujita, T. Yamauchi, I. Nishioka, *Chem. Pharm. Bull.* **1968**, 16, 1157.
- [35] Y. Shoyama, R. Oku, T. Yamauchi, I. Nishioka, *Chem. Pharm. Bull.* **1972**, 20, 1927.
- [36] M. Herkenham, A. B. Lynn, M. D. Little, M. R. Johnson, L. S. Melvin, B. R. De Costa, K. C. Rice, *Proc. Natl. Acad. Sci. U.S.A.* **1990**, 87, 1932.
- [37] R. Adams, D. C. Pease, C. K. Cain, J. H. Clark, *J. Am. Chem. Soc.* **1940**, 62, 2402.
- [38] L. E. Hollister, H. K. Gillespie, R. Mechoulam, M. Srebnik, *Psychopharmacology (Berlin)* **1987**, 92, 505.
- [39] R. Mechoulam, P. Braun, Y. Gaoni, *J. Am. Chem. Soc.* **1967**, 89, 4552.
- [40] P. J. Little, D. R. Compton, R. Mechoulam, B. R. Martin, *Pharmacol. Biochem. Behav.* **1989**, 32, 661.
- [41] R. Mechoulam, N. Lander, M. Srebnik, A. Breuer, M. Segal, J. J. Feigenbaum, T. U. Jarbe, P. Consroe, *NIDA Res. Monogr.* **1987**, 79, 15.
- [42] R. Mechoulam, J. J. Feigenbaum, N. Lander, M. Segal, T. U. Jarbe, A. J. Hiltunen, P. Consroe, *Experientia* **1988**, 44, 762.
- [43] R. Mechoulam, N. Lander, A. Breuer, J. Zahalka, *Tetrahedron: Asymmetry* **1990**, 1, 315.
- [44] W. A. Devane, F. A. Dysarz, M. R. Johnson, L. S. Melvin, A. C. Howlett, *Mol. Pharmacol.* **1988**, 34, 605.
- [45] S. Munro, K. L. Thomas, M. Abu-Shaar, *Nature* **1993**, 365, 61.
- [46] S. Galiegue, S. Mary, J. Marchand, D. Dussossoy, D. Carriere, P. Carayon, M. Bouaboula, D. Shire, G. Le Fur, P. Casellas, *Eur. J. Biochem.* **1995**, 232, 54.
- [47] I. Diaz-Laviada, L. Ruiz-Llorente, *Mini Rev. Med. Chem.* **2005**, 5, 619.
- [48] L. W. Padgett, *Life Sci.* **2005**, 77, 1767.
- [49] J. W. Huffman, *Mini Rev. Med. Chem.* **2005**, 5, 641.
- [50] C. B. Pert, S. H. Snyder, *Science* **1973**, 179, 1011.
- [51] R. Simantov, H. Snyder, *Proc. Natl. Acad. Sci. U.S.A.* **1976**, 73, 2515.
- [52] H. W. Kosterlitz, J. Hughes, *Life Sci.* **1975**, 17, 91.
- [53] W. A. Devane, A. Breuer, T. Sheskin, T. U. C. Jarbe, M. Eisen, R. Mechoulam, *J. Med. Chem.* **1992**, 35, 2065.
- [54] W. A. Devane, L. Hanuš, A. Breuer, R. G. Pertwee, L. A. Stevenson, G. Griffin, D. Gibson, A. Mandelbaum, A. Etinger, R. Mechoulam, *Science* **1992**, 258, 1946.
- [55] L. Hanuš, A. Gopher, S. Almog, R. Mechoulam, *J. Med. Chem.* **1993**, 36, 3032.
- [56] E. Frider, R. Mechoulam, *Eur. J. Pharmacol.* **1993**, 231, 313.

- [57] A. D. Sinor, S. M. Irvin, D. A. Greenberg, *Neurosci. Lett.* **2000**, *278*, 157.
- [58] D. Panikashvili, C. Simeonidou, S. Ben-Shabat, L. Hanuš, A. Breuer, R. Mechoulam, E. Shohami, *Nature* **2001**, *413*, 527.
- [59] R. Mechoulam, S. Ben-Shabat, L. Hanuš, M. Ligumsky, N. E. Kaminski, A. R. Schatz, A. Gopher, S. Almog, B. R. Martin, D. R. Compton, R. G. Pertwee, G. Griffin, M. Bayewitch, J. Barg, Z. Vogel, *Biochem. Pharmacol.* **1995**, *50*, 83.
- [60] T. Sugiura, S. Kondo, A. Sukagawa, S. Nakane, A. Shinoda, K. Itoh, A. Yamashita, K. Waku, *Biochem. Biophys. Res. Commun.* **1995**, *215*, 89.
- [61] L. Hanuš, S. Abu-Lafi, E. Fride, A. Breuer, D. E. Shalev, I. Kustanovich, Z. Vogel, R. Mechoulam, *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 3662.
- [62] A. C. Porter, J. M. Sauer, M. D. Knierman, G. W. Becker, M. J. Berna, J. Q. Bao, G. G. Nomikos, P. Carter, F. P. Bymaster, A. B. Leese, C. C. Felder, *J. Pharmacol. Exp. Ther.* **2002**, *301*, 1020.
- [63] S. M. Huang, T. Bisogno, M. Trevisani, A. Al-Hayani, L. De Petrocellis, F. Fezza, M. Tognetto, T. J. Petros, J. F. Krey, C. J. Chu, J. D. Miller, S. N. Davies, P. Geppetti, J. M. Walker, V. Di Marzo, *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 8400.
- [64] G. Milman, Y. Maor, S. Abu-Lafi, M. Horowitz, R. Gallily, S. Batkai, F. M. Mo, L. Offertaler, P. Pacher, G. Kunos, R. Mechoulam, *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 2428.
- [65] L. Offertaler, F. M. Mo, S. Batkai, J. Liu, M. Begg, R. K. Razdan, B. R. Martin, R. D. Bukoski, G. Kunos, *Mol. Pharmacol.* **2003**, *63*, 699.
- [66] M. Begg, P. Pacher, S. Batkai, D. Osei-Hyiaman, L. Offertaler, F. M. Mo, H. Liu, G. Kunos, *Pharmacol. Ther.* **2005**, *106*, 133.
- [67] N. R. Bachur, K. Mašek, K. L. Melmon, *J. Biol. Chem.* **1965**, *240*, 1019.
- [68] S. Conti, B. Costa, M. Colleoni, D. Parolaro, G. Giagnoni, *Br. J. Pharmacol.* **2002**, *135*, 181.
- [69] D. M. Lambert, S. Vandevorode, K. O. Jonsson, C. J. Fowler, *Curr. Med. Chem.* **2002**, *9*, 663.
- [70] T. Drmota, P. Greasley, T. Groblewski, to Astrazeneca, 'Screening assays for cannabinoid-ligand type modulators of GPR55', Pat. No. WO2004074844, 2004.
- [71] S. Ben-Shabat, E. Fride, T. Sheskin, T. Tamiri, M.-H. Rhee, Z. Vogel, T. Bisogno, L. De Petrocellis, V. Di Marzo, R. Mechoulam, *Eur. J. Pharmacol.* **1998**, *353*, 23.
- [72] S. Terrazzino, F. Berto, M. D. Carbonare, M. Fabris, A. Guiotto, D. Bernardini, A. Leon, *FASEB J.* **2004**, *18*, 1580.
- [73] M. Maccarrone, R. Pauselli, M. di Rienzo, A. Finazzi-Agro, *Biochem. J.* **2002**, *366*, 137.
- [74] M. Maccarrone, A. Cartoni, D. Parolaro, A. Margonelli, P. Massi, M. Bari, N. Battista, A. Finazzi-Agro, *Neuroscience* **2002**, *21*, 126.
- [75] F. Rodriguez de Fonseca, M. Navarro, R. Gómez, L. Escuredo, F. Nava, J. Fu, E. Murillo-Rodríguez, A. Giuffrida, J. LoVerme, S. Gaetani, S. Kathuria, C. Gall, D. Piomelli, *Nature* **2001**, *414*, 209.
- [76] B. Brito, R. Castro, A. Cabrera de Leon, *Lett. Drug Des. Discov.* **2006**, *3*, 741.
- [77] A. Ambrosini, G. Zolese, S. Ambrosi, L. Ragni, L. Tiano, G. Littarru, E. Bertoli, F. Mantero, M. Boscaro, G. Balercia, *Biol. Reprod.* **2006**, *74*, 659.
- [78] B. F. Cravatt, O. Prospero-Garcia, G. Siuzdak, N. B. Gilula, S. J. Henriksen, D. L. Boger, R. A. Lerner, *Science* **1995**, *268*, 1506.
- [79] L. O. Hanuš, H. M. Fales, T. F. Spande, A. S. Basile, *Anal. Biochem.* **1999**, *270*, 159.
- [80] A. S. Basile, L. O. Hanuš, W. B. Mendelson, *NeuroReport* **1999**, *10*, 947.
- [81] D. M. Lambert, V. Di Marzo, *Curr. Med. Chem.* **1999**, *6*, 757.
- [82] J. D. Leggett, S. Aspley, S. R. G. Beckett, A. M. D'Antona, D. A. Kendall, D. A. Kendall, *Br. J. Pharmacol.* **2004**, *141*, 253.
- [83] P. M. Hoi, C. R. Hiley, *Br. J. Pharmacol.* **2006**, *147*, 560.
- [84] A. Saghatelian, S. A. Trauger, E. J. Want, E. G. Hawkins, G. Siuzdak, B. F. Cravatt, *Biochemistry* **2004**, *43*, 14332.
- [85] A. Saghatelian, M. K. McKinney, M. Bandell, A. Patapoutian, B. F. Cravatt, *Biochemistry* **2006**, *45*, 9007.
- [86] ISI Web of Knowledge, Web of Science (July 22, 2007).

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