Cannabinoids, endogenous ligands and synthetic analogs

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The investigation of natural and synthetic cannabinoid ligands, including (–)-Δ^9-tetrahydrocannabinol, cannabinol, cannabidiol, HU-210, HU-211, CT3, CP 55,940, WIN 55,212-2, SR 14,1716A, anandamide, 2-arachidonoylglycerol, and numerous novel analogs, has led to important findings that have contributed to a better understanding of the role of these compounds in physiological processes. Their potential use for medicinal purposes is also better understood as a result.

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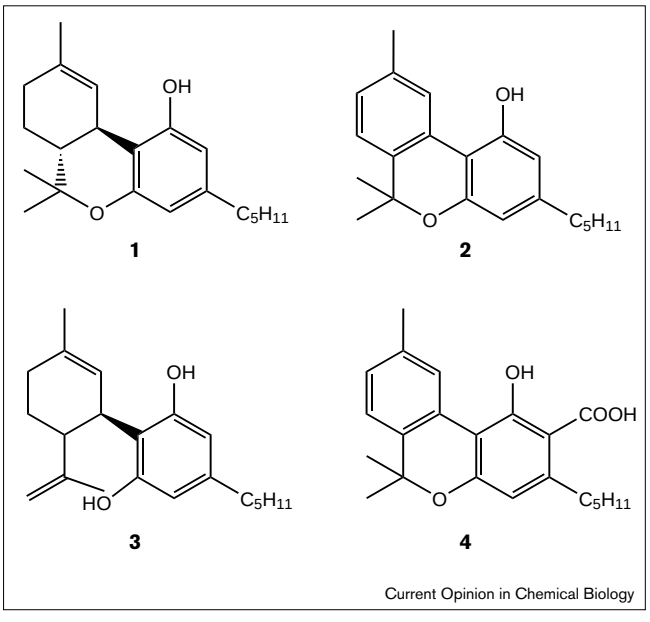
Abbreviations

2-AG	2-arachidonoylglycerol
AEA	anandamide (*N*-arachidonoylethanolamine)
CBR	cannabinoid receptor
THC	(–)-Δ^9-tetrahydrocannabinol

Introduction

Marijuana (cannabis), a crude preparation obtained from *Cannabis sativa*, has been known for millennia for its use as both a recreational psychoactive drug and for its therapeutic properties. The systematic study of cannabis and its components, a group of over 60 compounds known as cannabinoids, was only initiated in the 1960s, mostly as a result of the serious social implications of marijuana. Isolation, elucidation of structures and stereochemistry, total synthesis, metabolism, pharmacology and physiological effects of cannabinoids were followed in the 1980s and 1990s by identification and cloning of the specific cannabinoid receptors (CBRs) located in the central nervous system (CB1) and the periphery (CB2) and of the endogenous cannabinoid ligands. Studies of various aspects of CBRs and their ligands, development of several potent and selective agonist and antagonists to the CBR, and the study of potential medicinal uses has opened new avenues for cannabinoid research, placing it into the mainstream of medicinal chemistry. Although a large number of scientists have been involved with this work, the contribution of Raphael Mechoulam to the development of cannabinoids is quite remarkable. Here, I present recent advances in the cannabinoid field, selected from an impressive volume of literature published in 1998.

Figure 1



Natural cannabinoids. **1**, THC; **2**, cannabinol; **3**, cannabidiol; **4**, cannabinolic acid.

Cannabinoid receptors

The pharmacological, physiological and clinical implications of the discovery of CBRs [1•] their endogenous agonists [2] and their relationship with immunity [3•] have been reviewed elsewhere. Immunohistochemical distribution studies in the rat central nervous system mapped the localization of CB1 to the brain, spinal cord and within individual cells [4,5]. Various studies related to CB1, including its cellular regulation and distribution [6], the role of cannabinoids and receptors in modulating GABAergic neurons and GABA (γ-aminobutyric acid) uptake [7], disruption of the modulation of ion channels, and implicit activities of cannabinoids through CB1 phosphorylation [8] have been reported.

Natural cannabinoids

Marijuana and its main components — the psychoactive (–)-Δ^9-tetrahydrocannabinol (THC) (**1**, Figure 1), and the nonpsychotropic cannabinol (**2**), cannabidiol (**3**) and cannabinolic acid (**4**) — have been of continuous interest. Because of its traditional use as an illicit recreational drug and its notorious side effects, the medical use of cannabis (i.e. as an analgesic, antiemetic, antiglaucomic, etc.) continues to be debated and remains a controversial issue. In several countries, militants to the marijuana cause succeeded in imposing the legality of use of both the safer and scientifically more sound single cannabinoids, such as THC (Dronabinol, Marinol® [Unimed Pharmaceuticals, USA]) and Nabilone, and of the inexpensive, nonstandardized cannabis. The general consensus of opinion is that, in spite of the risks involved, marijuana should be available for patients who do not respond adequately to currently available therapies [9–12].

The effects of cannabis on brain and immune function have been reviewed [13,14]. Cumulative reports indicating that THC alters resistance to infection, link marijuana use to increased susceptibility to infection [15]. It was suggested that neurotoxicity of THC, which causes apoptosis (programed cell death) of hippocampal neurons, may be responsible for some of the memory deficit caused by cannabinoids [16]. Recently, the mesolimbic dopamine system has been implicated in the long-term aversion consequences of withdrawal from major drugs of abuse. Evidence that natural and synthetic cannabinoids share with other drugs of abuse the ability to stimulate, in a dose-dependent manner, mesolimbic dopaminergic neurons, which is an effect mediated by CB1, helps to explain the addictive properties of marijuana [17].

Cannabinoids, and particularly THC, are capable of inhibiting nociception (pain transmission) by interacting with CBRs. The potency of morphine anti-nociception in mice was significantly increased by some cannabinoids through a CBR-based mechanism [18].

Hampson *et al.* [19•] demonstrated that both THC and nonpsychotropic cannabidiol protect neurons in rat cortical cultures against toxic levels of the excitatory amino acid neurotransmitters. This activity, which is not related to the CBR but rather to the antioxidant capacity of these compounds, suggests that cannabidiol may be used as therapeutic agents for the treatment of oxidative neurological disorders such as cerebral ischemia.

Further evidence regarding the complex pharmacology of natural cannabinoids has been provided using a functional *in vitro* assay [20•]. A method was recently developed to examine receptor activation of G proteins in brain slices or membranes by stimulating the binding of the hydrolysis-resistant GTP analog [^{35}S]GTP-y-S with agonists. Results indicated that cannabinoid agonists are able to stimulate [^{35}S]GTP-y-S binding on slices or membranes. This study, using [^{35}S]GTP-y-S binding on rat cerebellar homogenate as an index of CBR activation, indicated evidence for a partial agonist activity of THC and antagonist activity of cannabidiol on CB1 receptors.

Synthetic cannabinoids

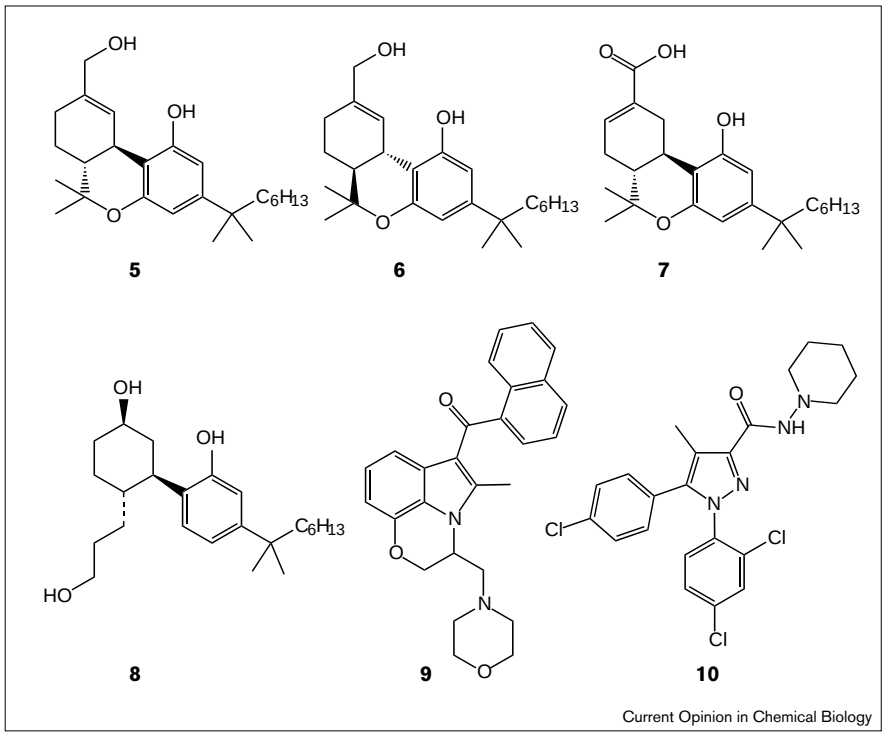
Several synthetic cannabinoids, including some that are closely related structurally to THC (such as the dimethylheptyl sidechain-containing HU 210 [**5**, Figure 2] and its nonpsychotropic optical enantiomer HU 211 [dexanabinol; **6**], nonpsychotropic carboxylic acid metabolites of cannabinoids such as dimethylheptyl-Δ^8-THC-11-oic acid, CT3 [**7**], the somewhat related [bicyclic] highly potent, selective CB1 agonists such as CP 55,940 [**8**], as well as the 'nonclassical' potent CB1 agonist aminoalkylindole-type WIN 55,212-2 [**9**] and the selective CB1 antagonist SR 141716A [**10**]) continue to be intensively studied. A number of novel synthetic cannabinoids have also been reported (**11**–**15**), and will be discussed below.

Established synthetic cannabinoids

Cannabinoids inhibit excitatory synaptic transmission, an effect that might represent a plausible cellular mechanism

Figure 2

Synthetic cannabinoids. **5**, HU 210; **6**, dexanabinol (HU 211); **7**, CT3; **8**, CP 55,940; **9**, WIN 55,212-2; **10**, SR 141716A.



underlying the cerebellar dysfunction they cause [21]. The effect of CBR activation and blockade on the propulsive activity in the mouse small intestine was assessed [22] by measuring the transit of an orally administered nonabsorbable marker. The CB1 agonist WIN55,212-2 inhibited, whereas the selective antagonist SR 141716A stimulated, the marker transit. Furthermore, a *per se* noneffective dose of SR 141716A reversed WIN55,212-2-induced reduction of the transit. These results suggest a role for CB1 receptors in the control of propulsive activity in the mouse small intestine.

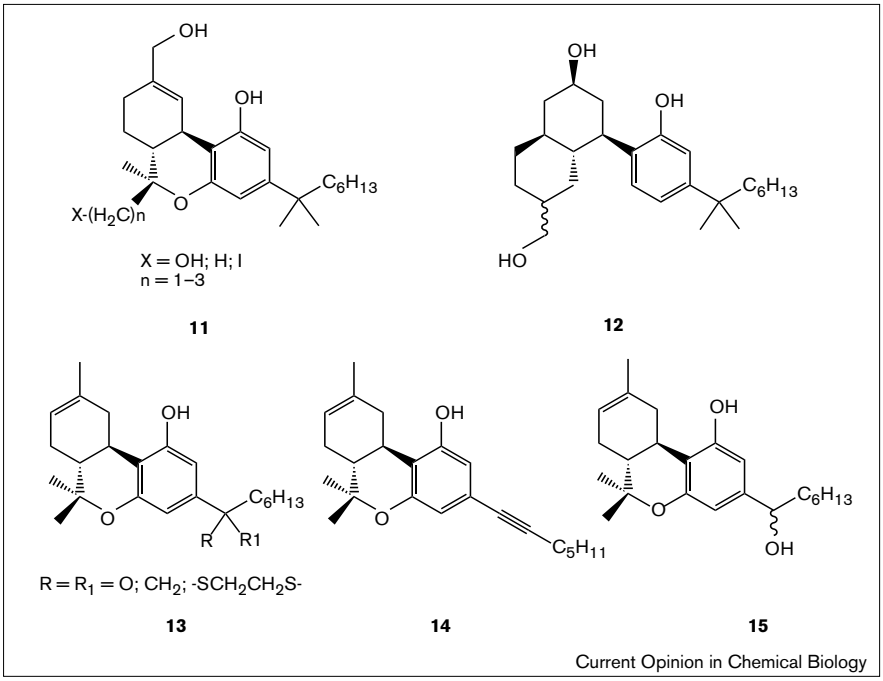
Moreover, the effects of four cannabimimetics (i.e. CP 55,940, WIN 55,212-2, **Δ**^{8}-THC [an isomer of THC] and AM 356 (a hydrolytically stable analog of anandamide) on detailed temporal parameters of operant responding was investigated [23]. The behavioural output during performance of a fixed ratio 5 schedule of reinforcement was recorded by a computer program that measured the response initiation time (IT) and the response duration. All four cannabinoids tested significantly suppressed operant lever pressing in a dose-dependent manner, the rank order of potencies observed being consistent with the rank order of affinities for CB1. All of them substantially increased average IT and also increased duration time. Together with other data, these results suggest that CB1 stimulation leads to motor effects that are associated with a suppression of lever pressing. Of further interest, compounds **8** and **9** protected neurons from excitotoxicity in a model of synaptically mediated neuronal cell death, suggesting that they might slow the progression of neurodegenerative diseases [24].

Examination of the *in vitro* binding characteristics of Δ^8-THC, and its metabolites, 11-OH-Δ^8-THC, 11-oxo-Δ^8-THC, and the nonpsychotropic Δ^8-THC-11-oic acid, indicated that active metabolites of Δ^8-THC competitively bind to the CBR as agonists [25]. *In vivo* studies [26] suggest, however, that the action of **10** (and **2**) at the human CBR are due to inverse agonism rather than antagonizsm of an endogenous agonist. Studies in Rhesus monkeys showed that **10** demonstrated selective and reversible antagonism of the behavioral effects of certain cannabinoids (THC, **9** and **21**) [27•].

Certain synthetic cannabinoids have been shown to elicit a variety of physiological effects. For example, the nonpsychotropic CT3 (**7**, Figure 2), is of particular interest as having significant analgesic [28•] and anti-inflammatory properties [29•]. The acute administration of the potent synthetic cannabinoid **5** to rats induces a set of endocrine alterations closely related to those described for THC, but at doses 50–200 times lower [30].

Importantly, clinical investigation of dexanabinol (**6**), a nonpsychotropic enantiomer of **5** continues successfully. The compound is currently developed by Pharmos Corporation (USA and Israel) as a neuroprotective agent for the treatment of brain damage associated with stroke, brain injury and cardiac arrest. Phase II clinical trials performed in Israel on severe head trauma patients have been considered as promising, although the number of patients was too small to lead to statistically significant results. These trials are important because currently there are no neuroprotective drugs available to limit brain cell death following injuries.

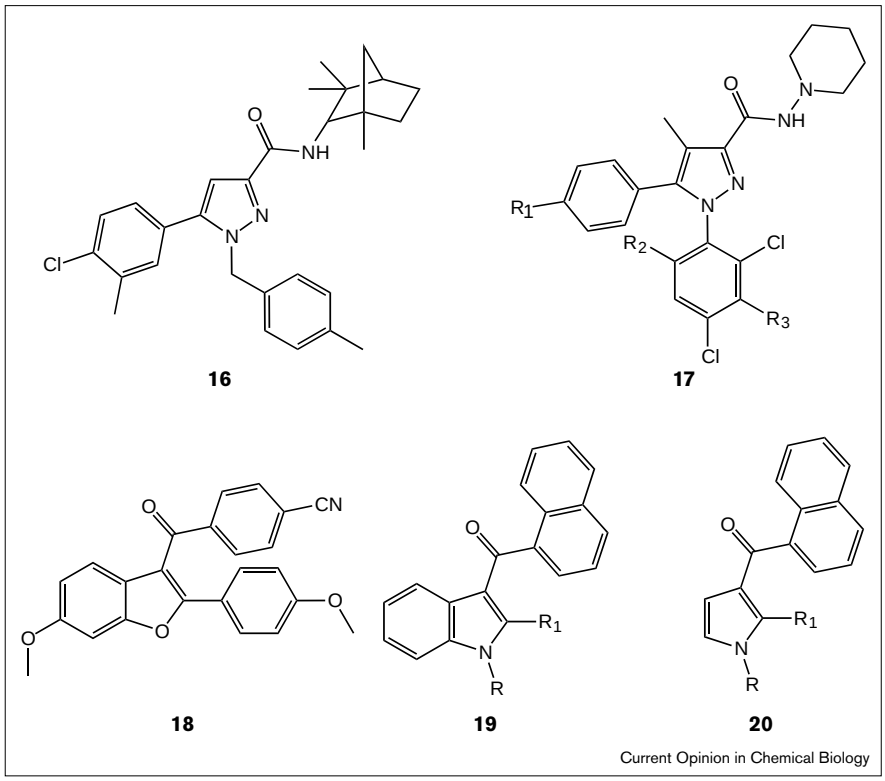
Figure 3



Classical/nonclassical hybrid cannabinoids and synthetic cannabinoids with modified sidechains. (See full text for details.)

Figure 4

Novel synthetic cannabinoids.
16, SR 144528; **17**, halogenated analogs of SR 141716A (R_1, R_2, R_3 = H, Cl, Br or I); **18**, LY 320135; **19**, **20**, indole- and pyrrole-derived cannabinoids (R = alkyl, alkenyl, etc.; R_1 = H, CH_3).



Several new cannabinoid ligands have been reported recently. Makriyannis and co-workers [31•] examined the stereochemical requirements of the southern pharmacophore region in the classical/nonclassical hybrid cannabinoids, containing the pyran B-ring and having modified C6β groups, for cannabimimetic activity (**11**, Figure 3). A conformational study of two nonclassical cannabinoids, CP 55,244 (which is pharmacologically 300-fold more potent than THC) and its diastereoisomer CP 97,587 (**12**), using NMR spectroscopy and molecular modeling, revealed that the orientation of the aliphatic hydroxyl group with respect to the third ring (D) is important for the pharmacophoric properties of these compounds [32]. A series of novel analogs of Δ^8-THC, in which the sidechain pharmacophores are conformationally more defined (**13**–**15**), showed nanomolar and subnanomolar affinities for CBR [33].

Novel synthetic cannabinoids

SR 144528 (**16**, Figure 4), developed by Sanofi Research as the first potent, highly selective and orally active antagonist for CB2, is expected to be a powerful tool to investigate the *in vivo* functions of the cannabinoid system in the immune response [34].

Receptor binding studies of a series of alternatively halogenated analogs of **10** (**17**) and classical cannabinoids suggested that **8** and other agonists interact with cannabinoid binding sites within the brain that are distinguishable from binding sites for **10**, its analogs and **3**. Moreover, structural modifications of **10** significantly altered their receptor affinity [35].

A new selective antagonist for CB1, LY 320135 (**18**, Figure 4), developed by Lilly, which reversed the effects of **21** (see Figure 5) and blocked the effects of **9**, is a lead compound for further development of novel, potent and selective cannabinoid antagonists [36].

A series of novel indole- and pyrrole-derived cannabinoids (**19**, **20**) were tested for CB1 binding affinity and underwent a battery of *in vivo* tests. It was discovered that receptor affinity and potency are related to the length of the carbon chain and that pyrrole-derived compounds were less potent than the corresponding indole derivatives [37].

Endocannabinoids

The endogenic cannabinoids are amides and esters of fatty acids, mainly arachidonic acid, which bind to CB1 and CB2 and mimic the pharmacological actions of THC. Metabolic aspects (e.g. biosynthesis, uptake, degradation) and the physiological role of the most important endocannabinoids, *N*-arachidonoylethanolamine (anandamide or AEA; **21**, Figure 5), 2-arachidonoylglycerol (2-AG) (**22**) and a number of synthetic analogs, have been intensively investigated.

Di Marzo [38•] reviewed the metabolic pathways of AEA and 2-AG, and the molecular mode of action of the metabolites and their implication in physiopathological responses. Degradation and loss of activity of AEA and 2-AG by enzymatic hydrolysis (via amido hydrolase) was further examined [39]. The rapid inactivation of 2-AG by neuronal and basophil-like cells through diffusion, hydrolysis and reacylation was inhibited by typical esterase inhibitors and by specific blockers of the fatty acid amide hydrolase, the enzyme that hydrolyzes AEA, suggesting the possibility that there is cross-regulation of 2-AG and AEA levels [40]. It was demonstrated [41] that several fatty acid glyceryl esters, not active by themselves, which accompany 2-AG, enhance its binding, lower 2-AG uptake into the cell and inhibit its hydrolysis. As a result, the *in vivo* effects of 2-AG are enhanced. This 'entourage effect' is an observation of general importance as natural products are usually administered as mixtures rather than in pure form.

The physiological role of endocannabinoids is not quite clear because of their rapid degradation *in vivo*. It appears, however, that they might act as a 'tuning system' for numerous physiological functions both in the central nervous system and in peripheral processes (such as modulation of neurotransmitter release/action at automatic and sensory fibres and the control of immunological, gastrointestinal, reproductive and cardiovascular performance. In the brain, this regulatory system may interact with thermoregulatory centers and might regulate perceptive (hearing, color, vision, taste) cognitive (sleep, long term potentiation, short-term memory) and motor (movement, coordination, posture, skeletal muscle tone) functions [38•].

Endocannabinoids in the central nervous system

Some effects of AEA have been related to the protective effects of cannabinoids on neurological disorders such as multiple sclerosis [42]. In addition to its role as a CBR ligand, AEA can also influence and modulate NMDA receptor-mediated neurotransmission [43].

Although AEA, SR 141716A and CP 55,940 compete for CB1 throughout the brain, SR 141716A does not block the pharmacological effects of AEA. This suggests that AEA may not produce all of its effects by direct interaction with CB1 [44]. Furthermore, a map of the brain nuclei responsive to AEA (which was injected intracerebroventricularly) showed that, in addition to CB1- and CB2-expressing nuclei, a new subset of nuclei that demonstrated no CBR expression showed responsiveness to AEA [45].

Mechoulam and co-workers [46] have shown that very low doses of AEA are stimulatory (rather than inhibitory) and actually reduce the effects of THC. This could explain some of the contradictory effects of AEA that have been documented, such as aggression versus sedation.

Endocannabionoids in the cardiovascular system

The significance of endocannabinoids in the cardiovascular system is still not adequately defined. The vasorelaxant activity of AEA can be explained by mediation of the nitric oxide (NO)- and prostanoid-independent component of endothelium-dependent relaxations. This controversial hypothesis is discussed by Randall and Kendall [47]and shall not be expanded upon here. Mechoulam *et al.* [48] found that stimulation of blood vessels with cholinergic agonists not only activate the release of NO but also of hypotensive 2-AG, suggesting that 2-AG might be a major constituent in blood pressure regulation mechanisms. Detection of CB1 and AEA in human vascular endothelial cells indicates that AEA might indeed play an important role in modulating vascular tone [49]. Other evidence suggests that the vasodilative effects of AEA are not related to CB1 but may be caused by arachidonic acid, resulting from the hydrolysis of AEA, which is converted to vasodilative eicosanoids [50].

Endocannabinoids in other systems

The finding that AEA potently and selectively inhibits proliferation of human breast cancer cells *in vitro* is of great interest; this occurs via a reduction of cells in the S phase of the cell cycle, rather than because of AEA toxicity or to apoptosis. Similar effects are manifested by (*R*)-methanandamide, AEA and HU 210, through CB1-mediated mechanisms [51•]. Importantly, AEA and the sleep-inducing agent oleamide (**24**, Figure 5) are autacoid suppressors of human breast cancer cell proliferation [52•].

It has also been demonstrated that AEA attenuates pain behavior produced by chemical damage to cutaneous tissue, by interacting with CB1-like receptors located (in the skin) [53•].

It appears that chocolate contains AEA, but the concentrations are too low to explain its somewhat addictive properties. On the other hand, 2-AG and **24** have been found in milk, which might have physiological significance [54].

Endocannabinoid analogs

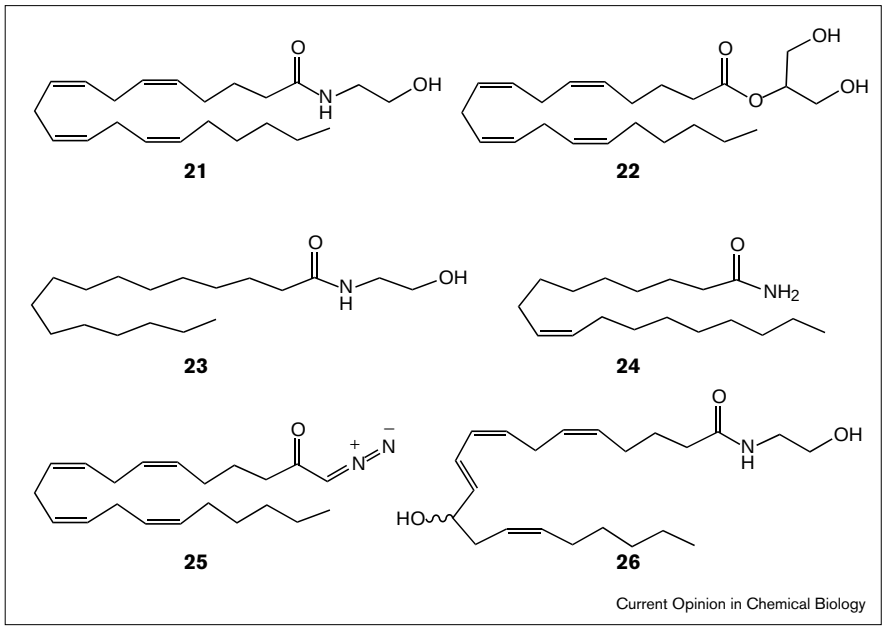
Some new endocannabinoids analogs have been reported: diazomethylarachidonyl ketone (**25**; Figure 5), designed as an irreversible inhibitor of AEA aminohydrolase, can be used to study the role of this enzyme in the termination of AEA activity [55]. An *in vitro* receptor binding study of oxygenated metabolites of AEA indicated the following: addition of a hydroxyl functionality at C12 of the arachidonate backbone of AEA, yielding 12(*S*)- and 12(*S*)-hydroxy-AEA (**26**; Figure 5), does not affect receptor binding, but increases its half-life; addition of an hydroxyl group to other positions on AEA affects its affinity for receptors; both the position of the hydroxyl groups and the configuration of the remaining double bonds determine affinity [56].

Conclusions

Some of the main achievements of the current cannabinoid research include the following: there is a better under-

Figure 5

Endocannabinoids. **21**, arachidonylethanolamine (AEA); **22**, 2-arachiodonoylglycerol (2-AG); **23**, palmitoylethanolamine; **24**, oleamide; **25**, diazomethylarachidonoyl ketone (DAAK); **26**, 12-hydroxy AEA.



standing of the mechanism of action of cannabinoids; there has been generation of more data regarding elucidation of the role of endocannabinoids in regulating various physiological processes; novel potent and specific CBR ligands have been studied. Finally, there has been an accumulation of new supporting evidence for potential therapeutical applications of some cannabinoids, including cannabidiol (as a neuroprotectant agent), CT3 (analgesic and anti-inflammatory), anandamide (analgesic and anticancer), SR-141716 (antipsychotic) and palmityl-ethanolaminide (analgesic).

Future research will probably continue to focus on the elucidation of cannabinoid metabolism and physiology and on the further development of some of the existing and newly designed CBR ligands for medicinal use.

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