Beyond the direct activation of cannabinoid receptors: new strategies to modulate the endocannabinoid system in CNS-related diseases

Andrea Chicca[a], Chiara Arena[a,b], Clementina Manera[b]

[a]Institute of Biochemistry and Molecular Medicine, National Center of Competence in Research TransCure, University of Bern, CH 3012 Bern, Switzerland; [b]Department of Pharmacy, University of Pisa, via Bonanno 6, 56126 Pisa, Italy

*To whom correspondence should be addressed. A.C.: *email address*: andrea.chicca@ibmm.unibe.ch; telephone: +41 (0) 31 6314125

Abstract

Endocannabinoids (ECs) are signalling lipids which exert their actions by activation cannabinoid receptor type-1 (CB_1) and type-2 (CB_2). These receptors are involved in many physiological and pathological processes in the central nervous system (CNS) and in the periphery. Despite many potent and selective receptor ligands have been generated over the last two decades, this class of compounds achieved only a very limited therapeutic success, mainly because of the CB_1-mediated side effects. The endocannabinoid system (ECS) offers several therapeutic opportunities beyond the direct activation of cannabinoid receptors. The modulation of EC levels *in vivo* represents an interesting therapeutic perspective for several CNS-related diseases. The main hydrolytic enzymes are fatty acid amide hydrolase (FAAH) for anandamide (AEA) and monoacylglycerol lipase (MAGL) and α,β-hydrolase domain-6 (ABHD6) and -12 (ABHD12) for 2-arachidonoyl glycerol (2-AG). EC metabolism is also regulated by COX-2 activity which generates oxygenated-products of AEA and 2-AG, named prostamides and prostaglandin-glycerol esters, respectively. Based on the literature and patent literature this review provides an overview of the different classes of inhibitors for FAAH, MAGL, ABHDs and COX-2 used as tool compounds and for clinical development with a special focus on CNS-related diseases.

Introduction

The endocannabinoid system (ECS) comprises different components including two G protein-coupled receptors, the type-1 (CB_1) and type-2 (CB_2) cannabinoid receptor, a class of arachidonoyl-derived lipids called endocannabinoids (ECs) which are produced on-demand from membrane phospholipid precursors and several enzymes involved in the biosynthesis and degradation of ECs. The more abundant and well-studied ECs are *N*-arachidonoylethanolamine (anandamide, AEA) which is a member of the large family of *N*-acylethanolamines (NAEs) and 2-arachidonoylglycerol (2-AG) that belongs to the monoacylglycerol family (1). Both ECs bind to CB_1 and CB_2 receptors although 2-AG behaves as a full agonist while AEA only partially activates the receptors (2). The biological activities of these lipid mediators are terminated upon cellular re-uptake and subsequent metabolism. Fatty acid amide hydrolase (FAAH) (3) and monoacylglycerol lipase (MAGL) (4) are the main enzymes involved in AEA and 2-AG hydrolysis, respectively. More recently, additional endocannabinoid-degrading enzymes have been also described. A second hydrolytic enzyme for AEA and NAEs was identified in humans (named FAAH-2) with a peculiar tissue distribution (kidney, liver, lung, ovary and heart) (5). Two serine hydrolases α,β-hydrolase domanin-6 (ABHD6) and α,β-hydrolase domain-12 (ABHD12) were recently identified as complementary 2-AG-degrading enzymes in the brain (6,7). Although MAGL accounts for most of the total 2-AG hydrolysis, ABHD6 and ABHD12 differ in the subcellular localization suggesting that they can play distinct roles in regulating 2-AG levels (8).

The COX-2-mediated oxygenation represents an alternative metabolic route for AEA and 2-AG which leads to the formation of prostaglandin-like molecules called prostamides and prostaglandin-glycerol esters, respectively (9,10). Although the hydrolysis is the most efficient degradation pathway for ECs, in certain tissues (e.g. brain, kidney) and conditions (e.g. inflammation), COX-2-mediated oxygenation can significantly contribute to terminate AEA and 2-AG effects. Furthermore, these oxygenated products possess peculiar and not yet fully understood biological actions during inflammation (11).

The direct activation of cannabinoid receptors results in several beneficial effects, in the brain and in the periphery, therefore numerous CB_1 and CB_2 agonists have been developed and tested *in vitro* and *in vivo*. Unfortunately, none of them reached an advanced stage of clinical development due to several drawbacks derived from direct and constant receptor activation which is responsible of numerous central nervous system (CNS)-related side effects (mainly via CB_1). On the other side, enhancing the endocannabinoid levels is expected to preserve the beneficial effects derived from the direct activation of CB receptors with more limited side effects (12,13). Therefore, alternative pharmacological strategies to raise the levels of AEA and 2-AG in tissues have been extensively investigated. These efforts led to the generation of many potent and selective inhibitors of endocannabinoid degradation which specifically target one of the main enzymes involved in AEA and 2-AG metabolism.

Here, we will review the main pharmacological targets of the ECS (FAAH, MAGL, ABHDs, COX-2 and the putative EMT (endocannabinoid membrane transporter) focusing on the different classes of inhibitors described in the literature and patent literature, highlighting the therapeutic applications and the potential translation aspects for CNS-related diseases and disorders (Fig. 1).

FAAH inhibitors

FAAH is a membrane-bound homodimer of 64 kDa subunits which belongs to the serine hydrolase family bearing an unusual Ser241-Ser217-Lys142 catalytic triad. FAAH is the main hydrolytic enzyme for NAEs and efficiently terminates the biological activities of AEA (3,14). The growing pharmacological interest in generating potent and selective FAAH inhibitors is connected to the observation that increasing levels of AEA in the tissue potentiate the activity of the ECS leading to many beneficial effects, like analgesia, anti-depressant, anxiolytic and anti-inflammatory (15). Unlike the direct activation of CB_1 receptors, FAAH inhibitors devoid the classical central side effects such as catalepsy, hypothermia, hypomotility and reinforcing effect (16). For these reasons, FAAH might represent an appealing therapeutic target for several central and peripheral diseases.

Many efforts have been made both by academia and pharmaceutical companies to develop potent and selective FAAH inhibitors. Among the numerous scaffolds described in the scientific literature and in patents, three main chemical families have been extensively studied: α-ketoheterocycles (17) (Fig. 2), carbamate-based (Fig. 3) (18) and urea-derived inhibitors (Fig. 4) (19).

The lead compound of α-ketoheterocycle-based inhibitors is OL-135 (**1**, Fig. 2) that blocks FAAH activity with an IC_{50} value of 4.7 nM (20). OL-135 and other α-ketoheterocycles act as reversible inhibitors through the formation of a hemiketal species involving the nucleophile Ser241 (21). Despite their low nanomolar potency *in vitro*, high doses of these compounds are necessary to enhance endocannabinoid signaling *in vivo*, probably because of the rapid metabolism in rodents. Starting from the lead compound OL-135, Boger's group at the Scripps Research Institute thoroughly investigated the influence of different heterocycles on inhibitors potency (22,23). The tetrahydronaphthalene derivative US2012106569 is one of the most stereoselective inhibitors, with the (*S*)-enantiomer (**2**, Fig. 2) to inhibit FAAH activity with a K_i value of 4 nM while the (*R*)-enantiomer (**3**, Fig. 2) showed a 60-fold lower potency. Administration of a single dose (50 mg/kg, po) of the more potent isomer to rats induced a significant accumulation of AEA, palmytoil ethanolamide (PEA) and oleoyl ethanolamide (OEA) in the brain that lasted for several hours. In a model of neuropathic pain, **1** (S-enantiomer) at the same dose exerted significant antinociceptive effects attenuating mechanical and cold allodynia (24).

Carbamate-based FAAH inhibitors contain the carbamic moiety that is usually used to inhibit serine hydrolases in an irreversible manner through the formation of a stable acyl-enzyme complex. URB597 (**4**, Fig. 3) (IC_{50} = 4.6 nM) can be considered the reference compound and the most investigated representative of this class of molecules (25–27). Indeed, URB597 dose-dependently inhibit FAAH activity in rat brain with an IC_{50} value of 0.15 mg/kg, leading to a significant increase of AEA and other NAEs, without interfering with 2-AG levels. Several pharmacological studies confirmed the anti-hyperalgesic and anti-allodynic effects of URB597 in different animal models of chronic pain without eliciting the typical central CB_1-mediated cannabimimetic effects (15,16,28–30).

URB597 has been recently included in an exploratory phase I clinical trial for the evaluation of new compounds with potential use in schizophrenia (NCT00916201) sponsored by the Central Institute of Mental Health in Mannheim (Germany). Nonetheless, the pharmacodynamics and pharmacokinetics profile of URB597 is not optimal since it inhibits other carboxylesterases and has a short half-life *in vivo* (31); therefore a second generation of carbamate-based FAAH inhibitors was developed. These new derivatives were obtained through the insertion of electron-donating polar groups at the phenyl ring attached to the carbamate oxygen, thus reducing the electrophilicity of the carbonyl (18). This new class of FAAH inhibitors includes URB694, which showed a negligible interaction with carboxylesterases, and an improved half-life *in vivo* compared to the parent compound URB597 (19). Recently, URB597/URB694-based inhibitors with an improved selectivity for FAAH and a restricted penetration to the central nervous system have been developed. The lead compound URB937 (**5**, Fig. 3), successfully blocked FAAH activity in the periphery without accessing the brain and thus representing a pharmacological tool for the treatment of pain, inflammation and immunological disorders (32).

Two novel classes of carbamate-based inhibitors were developed by Sigma-Tau Pharmaceuticals. The first class bears an enol carbamate template (ST-4070 (**6**, Fig. 3),) and ST-3899 (**7**, Fig. 3), IC_{50} value < 10 nM) (33), while the second is based on an oxime carbamate (ST-4020 (**8**, Fig. 2), IC_{50} < 10 nM) (34). These compounds were described as highly selective FAAH inhibitors over the main components of the ECS (i.e. CB_1, CB_2, $TRPV_1$, EMT, MAGL, at a concentration equal to 1000-fold their IC_{50} against FAAH). Among the enol carbamates, ST-4070 was the most active compounds against different models of neuropathic pain after oral administration (10-100 mg/kg) in rodents (35). Sigma-Tau also reported a class of carbamates structurally related to URB597. Compound ST-4068 (**9**, Fig. 3) blocks FAAH activity at subnanomolar concentrations (K_i = 0.49 nM) *in vitro* but exerts biological activities at 30 and 100 mg/kg (per os) in a mouse model of mechanical hyperalgesia (36). Other molecules belonging to this class of compounds were further studied by Butini et al. (37). The most potent inhibitor confirmed the antinociceptive activity after oral administration at the dose of 30 mg/kg without exhibiting any significant adverse effect indicating a favourable therapeutic index (36). Interestingly, these compounds behaved as reversible FAAH blockers, which is quite unexpected for carbamate-based inhibitors.

Due to their promising pharmacodynamics and pharmacokinetics profile, carbamate-based FAAH inhibitors were largely investigated by different pharmaceutical companies. Sanofi-Aventis reported different classes of compounds based on various *O*- and *N*-substituents including alkyl, piperazinyl, azetidinyl or thioazolyl (**10-11**, Fig. 3). These compounds were described as potent FAAH inhibitors *in vitro* with IC_{50} values in the nanomolar range and to exert antinociceptive effects *in vivo* upon oral administration of 1-30 mg/kg (38–45). A phase II clinical trial was undertaken to evaluate the FAAH inhibitor SSR-411298, for the treatment of major depressive disorders in the elderly patients (NCT00822744) and persistent cancer pain (NCT01439919). The long-lasting effects of carbamate-based inhibitors in elevating AEA levels *in vivo*, has encouraged researchers to further investigate other classes of irreversible FAAH inhibitors. Although the urea function is not usually considered a good

moiety to bind serine hydrolases because of its low reactivity (46), some pharmaceutical companies designed and developed urea-based FAAH inhibitors. Indeed, it was shown that with the introduction of a suitable leaving group the urea is converted into a more reactive group (e.g. tetrazole moiety), which can covalently bind the catalytic site of the enzyme (47). Pfizer thoroughly investigated this class of inhibitors generating valuable tool compounds and drug candidates. PF-3845 (**12**, Fig. 4) is a potent, selective and irreversible FAAH blocker (K_i = 0.23 μM) which covalently binds to the Ser241 at the catalytic site, resulting in a prolonged elevation of AEA levels in the brain and plasma in rats (48). PF-3845 reverted the lipopolysaccharide (LPS)-induced tactile allodynia in mice (49). Furthermore, PF-3845 promoted neuronal survival, attenuated inflammation and improved functional recovery in mice with traumatic brain injury (50) and persistently reduced inflammatory pain in rats through a cannabinoid receptor-dependent mechanism (48). As a further step, Pfizer, optimized the urea-based scaffold obtaining the compound PF-04457845 (**13**, Fig. 4), whose improved potency derived from the double bond between the biphenyl ether and the piperidine moiety and substitution of the pyridyl group with 3-aminopyridazine compared to its closely related analog, PF-3845 (51,52). A single oral administration of PF-04457845 produced potent antinociceptive effects in both inflammatory and non-inflammatory pain models in rats, without eliciting any effects in motility, catalepsy, and body temperature. Based on its great potency, selectivity and *in vivo* efficacy, combined with optimal pharmacokinetics properties, PF-04457845 was the first FAAH inhibitor to enter the clinical development for the treatment of pain disorders (52). Unfortunately, in a phase II clinical trial, it failed to induce analgesia in patients with osteoarthritic pain of the knee (53). Further evaluations of PF-04457845 are ongoing for the treatment of Tourette syndrome (NCT02134080), fear response (NCT01665573) and *Cannabis* withdrawal (NCT01618656). Pfizer also developed other series of urea-containing derivatives, including ether benzylidene piperidine (54), benzylidene 3-methylpiperidine derivatives (55), and rigid piperidines where the methylene group was replaced with a C4-spirocycle (representative compounds **14-18**, Fig. 4) (56). With few exceptions, these compounds were less potent than the reference compound PF-04457845. Other companies investigated urea-based compounds as FAAH inhibitors (57). Vernalis disclosed the structure of different azetidine urea derivatives. Among those, compound WO2009109743 (**19**, Fig. 4) showed a dose-dependent analgesic activity in a rat model of thermal pain after oral administration at 1-10 mg/kg (58). Janssen Pharmaceuticals also reported several patents covering heteroaryl-substituted ureas (59,60) exemplified by compound US2009062294 (**20**, Fig. 4), which inhibits human and rat FAAH in the nanomolar range but exerting only mild effects in a thermal injury model when administered orally to rats at 10 and 20 mg/kg (61). Janssen developed a class of spirocyclic diamine ureas, which potently inhibit FAAH activity. Within this class, JNJ-42119779 (**21**, Fig. 4) (62) showed antinociceptive effects in rodent model of neuropathic pain at 20 and 60 mg/kg, per os (63).

Other miscellaneous scaffolds were reported *in vitro* and *in vivo* as potent and selective FAAH inhibitors. Among them, some imidazole, oxazole and pyrrole-based compounds were developed by Merck and reported to inhibit FAAH activity with low nanomolar and subnanomolar potencies (**22-25**,

Fig. 5**)** (64–67). MK-4409 (**26**, Fig. 5) has been recently reported as a potent and selective, reversible, noncovalent FAAH inhibitor and showed excellent efficacy in numerous pain models in mice. In addition, no cognitive effects were observed for this brain permeable FAAH inhibitor. Based on its promising preclinical profile, MK-4409 was accepted as a lead candidate for further development in human clinical trials (68). Ironwood Pharmaceuticals patented several compounds as FAAH inhibitors. The indole ketoamide derivative MM-433593 (**27**, Fig. 5) is a highly potent and selective inhibitor with potential utility as an orally available treatment for pain, inflammation, and other disorders (69,70). Infinity Pharmaceuticals identified some isoaxazoline heterocycle-based molecules (some examples are **28-30**, Fig. 5 (71)) as novel covalent inhibitors for FAAH. The 5-(4-hydroxyphenyl)pentanesulfonyl fluoride (AM3506, **31**, Fig. 4) exhibited potent and selective inhibition of both rat and human FAAH. Rapid dilution assays and mass spectrometry analyses suggested that the compound is a covalent ligand which irreversibly blocks FAAH activity (72). Despite its potential therapeutic effects, AM3506 produced THC-like impairments in a rodent model of working memory (73). WOBE492 (**32**, Fig. 5) and WOBE491 (**33**, Fig. 5) are examples of *N*-alkylcarbamate inhibitors which have been developed starting from alkylamides present in different *Echinacea* species. These compounds showed an unusual potency for FAAH inhibition with IC_{50} values in the picomolar range (74).

MAGL inhibitors

MAGL is a soluble and ubiquitous enzyme mainly found associated to the inner leaflet of the plasma membrane. MAGL is responsible for the hydrolysis of monoacylgylcerols, being the main degrading enzyme for 2-AG *in vitro* and *in vivo*. Indeed, it has been shown in several animal models that MAGL regulates 2-AG levels in the brain and in peripheral tissues (4). The crystal structure of human MAGL was solved in 2010 (75) showing that the enzymatic architectures presents the hallmark of the α,β hydrolases superfamily. A cap domain, which varies much more among the members of this superfamily, covers the structurally conserved active site. Buried below the cap is the catalytic triad, made up of residues Ser122, Asp239 and His269, which is typical of the serine hydrolase family. An oxyanion hole stabilizes the tetrahedral anionic intermediate during hydrolysis and allows the interaction with cellular membranes creating a lipophilic/hydrophilic environment optimal for the accommodation of monoacylglycerols in the catalytic site (75).

Inside the catalytic site, targeting Cys201 and Cys242 provides a possible mean to regulate MAGL activity as it was exploited with the first generation of inhibitors (octhilinone (**34**, Fig. 6) (76) and *N*-arachidonoylmaleimide (**35**, Fig. 6) (77). An alternative target is the serine122 of the catalytic triad. Many recent carbamate-based inhibitors covalently bind this aminoacid residue as for example JZL184 (**36**, Fig. 6) (78), JZL195 (**37**, Fig. 6) (12), CAY10499 (**38**, Fig. 6) (79), KML-29 (**39**, Fig. 6) (80), MNJ-110 (**40**, Fig. 6) (81) and SAR127303 (**41**, Fig. 6) (82). Other inhibitors of miscellaneous structures include the β-lactone OMDM-169 (**42**, Fig. 7) (83), the α-keto oxiadozole **43**, Fig. 7) (84), the tetrazolcarboxamide AM6701 (**44**, Fig. 6) (85) the urea-based compounds (**45**, Fig.7) (SAR-629) (86), (compounds **46-48**, Fig. 7) (87–89), ML30 (**49**, Fig. 7) (90), JJKK-048 (**50**, Fig. 7) (91) the

natural-derived triterpenoid β-amyrin (**51**, Fig. 7) (92), euphol (**52**, Fig. 7) and pristimerin (**53**, Fig. 7) (93) and the recently identified benzodioxole derivative (**54**, Fig. 7) (94). The pharmacology of MAGL inhibitors in the CNS resembles most of the beneficial and detrimental effects associated to the direct activation of CB_1 receptors unlike the blockage of FAAH. Long et al., showed that JZL184 at the dose of 16 mg/kg increases the brain level of 2-AG by a factor 8 upon a single i.p. injection and induces analgesia, hypomotility and hypothermia in mice (95). Systemic administration of JZL184 at high doses (≥ 16 mg/kg) reduced nociception behavior in models of neuropathic (96) and inflammatory (97) pain and exhibited anxiolytic effects in the murble burying assay (98). Similarly, KML-29 (**39**, Fig. 6) showed CB_1-dependent antinoniceptive effects in the chronic constriction of sciatic nerve model of neuropathic pain and the carrageneen-mediated inflammatory pain (99) Unlike for JZL184, Ignatowska-Jankowska and colleagues reported that KML-29 does not elicit cannabinomimetic effects in the tetrad test at 40 mg/kg in C57BL/6J mice (99). More recently, Pasquarelli et al. investigated the changes in lipid levels in the brain and peripheral tissues upon injection of KML-29 at 0.5-10 mg/kg. In this work, the authors showed that lower doses of the inhibitor (5-10 mg/kg) are needed to increase the levels of 2-AG in the brain compared to Ignatowska-Jankowska et al. (20-40 mg/kg). Moreover, Pasquarelli et al., reported mild KML-29-induced cannabimimetic effects at 10 mg/kg, such as hypothermia, analgesia and hypomotility (100).

Recently, the structurally distinct carbamate SAR127303 (**41**, Fig. 6) confirmed the role of potent and selective MAGL inhibition as antinociceptive treatment (1-3 mg/kg per os), which is associated with worrying CB_1-dependent side effects such as the dose-dependent learning impairment and memory disruption (82). On the other hand other studies indicated beneficial effects in learning and memory upon genetic and pharmacological blockage of MAGL activity. Recently, it was shown that JZL184 improved synaptic plasticity and memory in a mouse model of Alzheimer's disease (101). Furthermore, MAGL-deficient mice also exhibited increased synaptic plasticity and memory (102), suggesting that disruption of MAGL activity could positively affect higher brain functions. Lysenco et al. also reported that MAGL inhibition improves the behavior and brain functions of Ts65Dn mice, a genetic model of Down Syndrome (103).

The pharmacological effects induced by potent and selective inhibition of MAGL activity turns into CB_1 functional antagonist upon chronic treatment. Scholsburg et al, showed that repeated administration of JZL184 for 6 days produces tolerance to its antinociceptive effects as a consequence of CB_1 receptor desensitization (104). Mice treated repeatedly with JZL184 at high doses (16-40 mg/kg) displayed cross-tolerance to the antinociceptive effects induced by the CB_1 agonists WIN55,212-2 and Δ^9-THC in neuropathic and thermal pain models (104,105). Similarly, MAGL-deficient mice exhibited elevated 2-AG levels in the CNS and a subsequent long-term activation of CB_1 receptors leading to receptor desensitization and unaltered pain sensitivity in neuropathic and inflammatory mouse models (106). The administration of MAGL inhibitors at low doses retained the beneficial CB_1-mediated effects even after repeated administrations without leading to cross-tolerance and functional antagonism at CB_1. Several independent studies reported anxiolytic, antinociceptive,

anti-nausea, anti-epileptogenic, anti-depressive and anti-inflammatory effects of acute and chronic administration of MAGL inhibitors at low doses (97,105,107–112). Abide Pharmaceuticals recently started a phase I clinical trial testing safety, pharmacokinetics and pharmacodynamics of ABX-1431, a new first-in-class MAGL inhibitor.

An alternative approach to avoid receptor desensitization consisted in designing potent and reversible MAGL inhibitors. Apart from three natural-derived terpenoids (β-amyrin (**51**), euphol (**52**) and pristimerin (**53**), (Fig. 7) (92,93), only one study has recently described a benzodioxole derivative (**54**, Fig. 7) as a reversible, time-independent MAGL inhibitor with an IC_{50} value of 240 nM and 75-fold selectivity over FAAH inhibition (94). Upon 3-weeks daily i.p. administration at 5 mg/kg, the compound ameliorated disease progression in the experimental autoimmune encephalomyelitis (EAE)-induced multiple sclerosis mouse model without undergoing tolerance and eliciting catalepsy, hypomotility and hypothermia (94).

MAGL inhibitors provide many of the beneficial effects observed with direct cannabinoid receptor agonists. Nonetheless, the clinical development of irreversible inhibitors has been hindered due to cannabimimetic side-effects and the development of tolerance upon repeated administrations at high doses. Additional studies showed that the use of MAGL inhibitors at low doses leads to a mild increase of 2-AG levels in the brain which still retain the therapeutic effects but do not trigger receptor desensitization and tolerance after chronic treatment. Similarly, the inhibition of MAGL activity by using a potent and reversible compound produced positive effects upon 21 days of treatment. These alternative pharmacological strategies can pave the way towards the exploitation of MAGL as therapeutic target for clinical development.

ABHD6

ABHD6 and ABHD12 are other members of the serine hydrolase family that are involved in 2-AG hydrolysis. Both ABHDs are integral membrane proteins whose catalytic site is predicted to face the intracellular and extracellular space for ABHD6 and ABHD12, respectively (8). MAGL is the most efficient 2-AG degrading enzyme being responsible for 80-85% of the total hydrolysis in the brain, where ABHDs account for the remaining 15-20% (7,8). Since these three serine hydrolases have different distributions and subcellular localizations, it was supposed that they could play distinct roles in controlling the duration of action and fate of 2-AG in the brain (6). Full and prolonged inhibition of MAGL activity produces an “overflow” of 2-AG which results in psychotropic effects and cannabinoid receptor desensitization (8,106,113). Therefore a milder modulation of the enzyme activity might be more indicated especially for chronic treatments. This could be achieved by using partial or reversible MAGL inhibitors (see previous paragraph) or selectively blocking ABHDs activity. The inhibition of ABHD6 leads to a moderate increase of 2-AG in the brain, thus suggesting that selective inhibitors may provide an alternative therapeutic option which lacks CNS-related side effects (114–116).

At present, only a limited number of ABHD6 inhibitors have been described. The first potent and selective ABHD6 inhibitor, WWL70 (**55**, Fig. 8) (IC_{50} = 70 nM) was discovered in Cravatt’s group

(117). Marrs and colleagues identified UCM710 (**56**, Fig. 8) as a dual inhibitor of ABHD6 and FAAH (115). Some other non-specific serine hydrolase inhibitors such as methyl arachidonyl fluorophosphonate (MAFP) (**57**, Fig. 8), tetrahydrolipstatin (THL) (**58**, Fig. 8), RHC-80267 (**59**, Fig. 8), and the triterpene pristimerin (**53**, Fig. 8) have been identified *in vitro* as ABHD6 inhibitors (118). Recently, Cravatt and colleagues identified a novel carbamate-based compound, WWL123 (**60**, Fig. 8), (119) along with some triazole urea derivatives like KT195 (**61**, Fig. 8), (120,121) as selective and potent ABHD6 inhibitors.

The inhibition of ABHD6 has been suggested to regulate the 2-AG signaling in the brain and shown to be effective in different animal models (7,122–124). In two independent works, Zhang's group investigated the protective effects of WWL70 in a mouse model of traumatic brain injury (TBI) (122), and EAE-induced multiple sclerosis (123).

TBI is a form of brain injury caused by sudden damage of the brain, and it is characterized by an initial insult followed by the secondary injury associated with excitotoxicity, inflammation, neuronal death and oxidative stress (125,126). In a TBI model, the post-insult chronic treatment with WWL70 at 10 mg/kg improved motor coordination and restored TBI-induced deficits in working memory in a CB_1-mediated mechanism (122). WWL70 treatment also reduced the lesion volume in the cortex and neurodegeneration in the dentate gyrus in a CB_1-/CB_2-dependent manner (122). In 2015, the same group assessed the role of ABHD6 inhibition in a neuroinflammatory mouse model (123). Leucocyte migration into the brain is an early event of neuroinflammation (127–129), and it is regulated by the increased expression of the leukocyte adhesion molecule (ALCAM) (130). Zhang and colleagues demonstrated that ALCAM overexpression in EAE mice was strongly reduced as well as the production of pro-inflammatory cytokines (TNF-α and IL-1β) by WWL70 treatment (10 mg/kg) (123). These effects were lost upon pre-treatment with a CB_2 selective antagonist and in CB_2-deficient mice, thus suggesting that the neuroprotection occurs via an indirect activation of CB_2 receptors in microglia/macrophage cells (i.e. increasing 2-AG levels) (123). In 2014, Naydenov and colleagues demonstrated that blocking ABHD6 exerts protective effects in an animal model of epileptic seizures (124). Indeed, the selective ABHD6 inhibitor WWL123 decreased pentylenetetrazole (PTZ)-induced epileptiform seizures and spontaneous seizures in R6/2 mice (124). While this effect resulted CB_1- and CB_2-independent, it was completely abolished by adding a sub-convulsive dose of the $GABA_A$ antagonist picrotoxin (124), thus suggesting that increased levels of 2-AG might exert beneficial effects by directly interacting with this receptor in agreement with the 2-AG positive allosteric properties recently described at the $GABA_A$ receptor (131,132). $GABA_A$ and ABHD6 are both expressed in post-synaptic neurons, where the 2-AG signalling might be locally amplified (124). Importantly, chronic inhibition of ABHD6 activity was not associated either with psychomotor and cognitive effects or tolerance, which are both typical features of prolonged and complete MAGL inhibition (124).

Therefore, ABHD6 inhibition might represent a novel approach to achieve a fine-tuned modulation of 2-AG signalling in restricted brain areas with potential therapeutic relevance in neurodegenerative and neuroinflammatory disorders and as a novel safe antiepileptic strategy. Although some inhibitors have

been generated, more work is still needed to develop more druggable compounds for potential clinical evaluation.

ABHD12

Since ABHD12 has been recently identified as a 2-AG hydrolytic enzyme which marginally contributes to its degradation in the brain (5-10 %), it was explored as a potential pharmacological target similarly to the ABHD6 (see above) (6,8). Subsequent genetic studies performed in humans demonstrated that mutations in the *Abhd12* gene represent the main cause of a progressive, autosomal-recessive neurodegenerative disease, first discovered in a Norwegian family (133). This disorder was named polyneuropathy, hearing loss, ataxia, retinitis pigmentosa, and cataract, or PHARC (134).

The minor role of ABHD12 in 2-AG hydrolysis in contrast with the serious PHARC phenotype in the brain and eye suggests either that ABHD12 is of crucial importance only in certain cell types or that its major endogenous substrates is not 2-AG (133). With the goal of understanding the role that ABHD12 in the CNS, Blankman and coworkers generated ABHD12-deficient mice, as a model for studying PHARC disease discovering that these animals have significant elevations of a series of lysophosphatidylserine lipids, which were then identified as the major ABHD12 substrates (135). Although the bioactive functions of lysophosphatidylserines in the brain are not clear, the work of Blankmann and coworkes suggested that these lipids can play a relevant role in microglial functions and activation (133). As the role of ABHD12 in lysophosphatidylserines metabolism and 2-AG hydrolysis is still poorly characterized, selective inhibitors may represent a valid tool to further investigate the pharmacological role of this enzyme in the CNS. At present not many selective inhibitors of ABHD12 have been described. An initial profiling showed that the general lipase/serine hydrolase inhibitors MAFP (**57**, Fig. 8) and THL (**58**, Fig. 8) are relatively potent inhibitors of ABHD12, although being not selective (118). Recently, Parkkari and co-workers, identified a series of natural compounds and their synthetic derivatives as the first selective inhibitors for ABHD12 (136). These compounds structurally belong to the class of triterpenoids that are known to possess a wide-range of therapeutic actions. Some of these compounds behaved as reversible inhibitors with IC_{50} values in the submicromolar range, without any major cross-reactivity with the other components of the ECS (136). These data might support the development of the first pharmacophore model for ABHD12, which can be used in further studies aiming at the discovery of novel lead structures to generate more selective inhibitors.

Endocannabinoid membrane transport (EMT) inhibitors

The underlying molecular mechanisms of endocannabinoid membrane transport (i.e. release into the extracellular space and cellular uptake) are not yet completely understood. Among the different proposed mechanisms, a facilitated diffusion process mediated by a putative EMT is the most supported model by *in vitro* and *in vivo* evidence (for review see Nicolussi & Gertsch, 2015 (137). Recently, we showed that the EMT specifically mediates the bidirectional movement of both major

endocannabinoids over other related lipids (i.e. NAEs and monoacylglycerols), suggesting that it can represent an alternative target in the ECS (138). The pharmacology elicited by pure EMT inhibitors is expected to only partially overlap with FAAH and MAGL inhibitors. Unfortunately, the first generation EMT inhibitors (UCM707 (**62**), OMDM-2 (**63**), AM404 (**64**) and VDM11 (**65**), Fig. 9) that have been characterized *in vitro* and *in vivo* were not very potent (IC_{50} values in micromolar range) and exhibited a limited selectivity over the other components of the ECS (i.e. CB receptors, degrading enzymes, TRPV1 receptors, intracellular carrier proteins) leading to a biased evaluation of their pharmacology.

More recently, we described guineensine (**66**, Fig. 9) and BSL-34 (**67**, Fig. 9) , two natural-derived compounds, as potent and selective inhibitors of AEA and 2-AG uptake (IC_{50} in the nanomolar range) with a good selectivity over FAAH, MAGL and CB receptors (≥ 50 fold) (74,139). In mice, guineensine elicited indirect cannabimimetic effects including hypomotility, decreased body temperature, catalepsy and analgesia (2.5-10 mg/kg) indicating that by blocking the cell membrane transporter, both AEA and 2-AG levels are increased (12).

The possibility to handle selective inhibitors of AEA and 2-AG uptake which do not interact with any of the known metabolic enzymes or AEA-binding proteins, will strongly contribute to shed light on the functional role of the EMT and the possibility to exploit the modulation of EC trafficking across the plasma membrane as an alternative therapeutic approach. While excellent tool compounds have been synthesized for most targets within the ECS (31), novel and more potent (ideally low nanomolar) chemical probes are needed to ultimately study endocannabinoid membrane transport.

New pharmacological strategies to modulate the ECS

Endocannabinoids are arachidonoyl-containing molecules, making them suitable to be directly and indirectly involved in the generation of oxygenated lipids via COX-2-mediated oxygenation (i.e. prostaglandins). In the brain and spinal cord, COX-2 is constitutively expressed in neurons and plays a role in the early hyperalgesic response to tissue injury (140). Nomura et al. convincingly demonstrated that 2-AG represents the main source of free arachidonic acid in the brain and that the pharmacological and genetic blockage of 2-AG hydrolysis leads to a complete inhibition of LPS-induced PGE_2 formation unlike in phospholipase A2-deficient mice (141). MAGL blockade exerted protective effects against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of Parkinsonism, in which COX inhibitors are known to be neuroprotective. Pharmacologic or genetic inactivation of MAGL prevented MPTP-induced dopaminergic neuronal loss and dopamine reductions in the substantia nigra. Intriguingly, these neuroprotective effects were accompanied by the blockade of MPTP-induced increase of arachidonic acid, prostaglandins and pro-inflammatory cytokines in the brain (141).The COX-2-mediated oxygenation of AEA and 2-AG generates two families of prostaglandin molecules which bear the ethanolamide and the glycerol ester head group which are named prostaglandin-ethanolamides (PG-EAs, prostamides) and prostaglandin-glycerol esters (PG-GEs), respectively (9,10,142,143). Unlike arachidonic acid, AEA and 2-AG cannot be metabolized by the constitutive COX-1 isoform due to the strict requirement for a free carboxyl group in the substrate

(144). The biological activities of PG-EAs and PG-GEs are distinct from those of the corresponding PGs and are probably mediated by distinct receptors. The membrane target of $PGF_{2\alpha}$-EA (**68**, Fig. 9) and its analog bimatoprost (**69**, Fig. 10) was identified as a heterodimer of the wild type FP receptor and a particular splice variant (Alt4) of this receptor (145). Selective antagonists of this receptor (AGN204396 (**70**), AGN-21135 (**71**) AGN-21136 (**72**) , Fig. 10) blocked $PGF_{2\alpha}$-EA effects without altering those of $PGF_{2\alpha}$ (145–148). Among the pharmacological actions of FP-FP(Alt4) receptor activation, the hyperexcitation of spinal cord nociceptive neurons has been showed to contribute sustaining pain under conditions of inflammation (149). Recently, Ligresti et al. described that two of the $PGF_{2\alpha}$-EA antagonists (AGN-21135 (**71**) AGN-21136 (**72**) , Fig. 10) inhibit FAAH activity with low micromolar IC_{50} values (150). Since AEA exerts analgesic effects, whereas its COX-2 metabolite $PGF_{2\alpha}$-EA is hyperalgesic (149) AGN-211335 and AGN-211336 might result in synergistic effects. Indeed, both compounds increased AEA levels *in vitro* and exerted antinociceptive effects in the formalin-induced pain model in mice (150). COX-2 is a functional heterodimer with an allosteric and a catalytic monomer (151) and it was shown that some of the classic non-steroidal anti-inflammatory drugs (NSAIDs) inhibit the oxygenation of 2-AG with ≥100-fold lower IC_{50} values compared to arachidonic acid oxygenation, and were defined as COX-2 substrate-specific inhibitors (SSIs) (152). (*R*)-Flurbiprofen (**73**, Fig. 10) represents a typical example of this class of inhibitors that potently blocked endocannabinoid oxygenation with negligible effects on arachidonic acid metabolism (152). The morpholine derivative of indomethacin, LM-4131 (**74**, Fig. 10) was recently described as a COX-2 SSI and showed to selectively increase the levels of AEA and 2-AG in the brain over other related lipids (153). Additionally, LM-4131 induced CB_1-dependent anxiolytic effects without eliciting other unwanted cannabimimetic side effects. COX-2 SSIs might achieve synergistic effects by inhibiting the formation of pro-inflammatory prostamides and PG-GEs and increasing the levels of AEA and 2-AG. On the other hand, PG-GEs and PG-EAs do not mediate the same biological effects, with PGE_2-EA and PGD_2-GE being anti-inflammatory while $PGF_{2\alpha}$-EA and PGE_2-GE being pro-inflammatory and hyperalgesic (154,155). Indeed, using the selective PGD synthase inhibitor HQL79 (**75**, Fig. 9), Alhouayek et al., could show that PGD_2-GE exerts anti-inflammatory effects in human macrophages and in mice. In the same setting (*R*)-flurbiprofen, but not CB_1 and CB_2 antagonists counteracted the 2-AG-induced decrease of macrophage activation, suggesting that PGD_2-GE is the main "transducer" of 2-AG effects (154). In light of the different roles as pro- and anti-inflammatory cytokines it would be desirable to selectively modulate one of the biosynthetic enzymes or alternatively blocking the receptor interaction of specific PG-EAs or PG-GEs. The main open questions are related to the possibility to develop substrate-specific PG synthase inhibitors and to gain a thorough understanding of the target receptors of the main PG-EAs and PG-GEs.

Alternative strategies to modulate the ECS include the multi-target approach which consists in developing compounds that hit 2 or more components of the system. We recently reported a class of β-

caryophyllene-derived amides (**76**-**78**, Fig. 11) bearing interesting different polypharmacology features (156). The trishomo seco-caryophyllene propyl (**76**, Fig. 11) and 2-propenylamide (**77**, Fig. 11) behave as CB_2 receptor agonists and simultaneously inhibit FAAH activity thus potentially leading to synergistic effects. Slight modifications of the amide group by replacing the short alkyl chain of **76** and **77** with a vanillimide group (**78**, Fig. 11) led to a different polypharmacology. Indeed compound **78** simultaneously inhibit FAAH activity and COX-2-mediated oxygenation of ECs. Interestingly, the latter combination might be particularly relevant in tissues that constitutively express COX-2 (i.e. the brain) or under inflammatory conditions, where inhibiting FAAH activity can re-direct AEA metabolism toward the COX-2-mediated oxygenation, thus potentially generating contrasting effects (156). Along the same lines, recent preclinical data indicated that combining COX-2 and FAAH inhibition produces enhanced antinociceptive effects in a variety of pain models. Different groups showed that the dual inhibition of COX-2 and FAAH enzymes offers a novel therapeutic strategy to attenuate inflammatory and neuropathic pain states (157,158). Specifically, combination of sub-threshold doses of PF-3845 and diclofenac produced marked anti-allodynic effects in neuropathic and inflammatory pain models which are associated with increased AEA levels and decreased PG levels in the brain. Therefore, the development of dual FAAH/COX-2 inhibitors would be of great potential value as a therapeutic strategy to treat different types of pain. 6-methyl-pyridin-2-yl analogue of ibuprofen ("ibu-am5") (**79**, Fig. 11) (159,160), the corresponding amide analogue of flurbiprofen Flu-AM1 (**80**, Fig. 11) (157) and carprofen (**81**, Fig. 11) (161) are prototypes of this class of compounds. Recently, Gouveia-Figueira et al., described that the (*R*)-enantiomer of Flu-AM1 behaves as a COX-2 SSI similarly to the parent compound (*R*)-flurbiprofen (162). Together, these data support the development of dual FAAH/COX-2 inhibitors as a potential therapeutic strategy for the treatment of neuropathic and inflammatory pain states. Further investigations are needed to generate more efficacious and selective probes and potential druggable candidates with distinct chemical structures from the current FAAH inhibitors and NSAIDs.

A recent study investigated the effects of combining low doses of the MAGL inhibitor JZL184 with the COX-2 inhibitor diclofenac. In a mouse model of neuropathic pain, the combination showed to synergistically reduce chronic constriction injury-induced mechanical allodynia and additively reduced cold allodynia in a CB_1-dependent manner. Interestingly, the combination of drugs significantly decreases PGE_2 and $PGF_{2\alpha}$ levels but does not affect the levels of 2-AG in the spinal cord (163). Dual inhibition of COX-2 and MAGL activity might represent an alternative promising therapeutic approach for reducing neuropathic pain with minimal side effects.

The natural compound (4'-methoxy-5,3'-di-(2-propenyl)-biphenyl-2-ol (**82**, 4'-*O*-methylhonokiol , MH, Fig. 11) represents the prototype of a novel polypharmacology within the ECS. We and others reported that MH behaves as a potent and selective CB_2 agonist that elicits a concentration-dependent inhibition of intracellular Ca^{2+} release and forskolin-induced cAMP formation (164–166).

Recently, we reported that MH can specifically inhibit COX-2-mediated oxygenation of AEA and 2-AG at concentrations that also activate CB_2 receptors (165). Furthermore, MH dose-dependently

increased 2-AG levels in the brain without affecting either arachidonic acid or PGE_2 levels (165). In hippocampal neurons, COX-2 inhibition rather than FAAH blockage led to a prolonged depolarization-induced suppression of inhibition (DSI) suggesting a prominent role of 2-AG in the retrograde signal and COX-2-mediated oxygenation as relevant mechanism of reducing 2-AG levels at the synaptic level (167). Straiker et al. also reported the relevant role of COX-2 activity in reducing EC-mediated retrograde signalling (168). In animal models, low doses of MH produced weak anxiolytic effects (169) similar to the COX-2 SSI LM-4131 (153). MH also showed beneficial effects in an Alzheimer's disease model by reducing LPS-induced β-amyloid accumulation, pro-inflammatory cytokine production, and memory impairment (170,171). In addition to the direct activation of CB_2 receptors, MH also elevates 2-AG levels, and inhibits the formation of pro-inflammatory PG-EAs and PG-GEs. Therefore, synergistic effects for attenuating the inflammatory process are expected to represent one of the main underlying mechanisms of the neuroprotective actions exerted by MH, indicating that the double CB_2 activation and COX-2 SSI might represent a promising polypharmacology strategy to be further explored.

Conclusions

The endocannabinoid system offers several therapeutic opportunities for CNS-related disorders beyond the direct activation of cannabinoid receptors (Fig. 12). The inhibition of FAAH activity represents a safe and effective pharmacological strategy to treat neurological disorders. Potent and prolonged MAGL inhibition is associated to cannabimimetic effects and receptor desensitization upon repeated administrations. Nonetheless, recent studies showed that brain levels of 2-AG can be mildly increased by partially blocking MAGL activity (using low doses of covalent and reversible inhibitors) or inhibiting other serine hydrolases (ABHD6), which retain the beneficial effects but lack the unwanted side effects. One of the main drawbacks related to FAAH and MAGL inhibition is the non-specific increase of many bioactive lipids (NAEs and monoacylgylcerols) beyond AEA and 2-AG. The inhibition of endocannabinoid trafficking across the cell membrane specifically modulates AEA and 2-AG levels over other related lipids. Unfortunately, potent and selective inhibitors for this process are still missing. The inhibition of COX-2-mediated endocannabinoid oxygenation might represent a novel promising therapeutic option to augment AEA and 2-AG levels and to prevent the formation of bioactive prostamides and prostaglandin-glycerol esters. The simultaneous inhibition of two or more targets within the endocannabinoid system might offer a superior pharmacological profile over the single treatment. Several possibilities of multi-target approach have been proposed and investigated *in vitro* and *in vivo* and represent one of the most promising future therapeutic options to modulate the endocannabinoid system.

References

1. Di Marzo V. Targeting the endocannabinoid system: to enhance or reduce? Nat Rev Drug Discov 2008;7(5):438–55.

2. Hillard CJ. Biochemistry and pharmacology of the endocannabinoids arachidonylethanolamide and 2-arachidonylglycerol. Prostaglandins Other Lipid Mediat 2000;61(1-2):3–18.
3. Cravatt BF, Giang DK, Mayfield SP, Boger DL, Lerner RA, Gilula NB. Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. Nature 1996;384(6604):83–7.
4. Dinh TP, Carpenter D, Leslie FM, Freund TF, Katona I, Sensi SL, et al. Brain monoglyceride lipase participating in endocannabinoid inactivation. Proc Natl Acad Sci U S A 2002;99(16):10819–24.
5. Wei BQ, Mikkelsen TS, McKinney MK, Lander ES, Cravatt BF. A second fatty acid amide hydrolase with variable distribution among placental mammals. J Biol Chem. 2006;281(48):36569–78.
6. Blankman JL, Simon GM, Cravatt BF. A comprehensive profile of brain enzymes that hydrolyze the endocannabinoid 2-arachidonoylglycerol. Chem Biol. 2007;14(12):1347–56.
7. Marrs WR, Blankman JL, Horne EA, Thomazeau A, Lin YH, Coy J, et al. The serine hydrolase ABHD6 controls the accumulation and efficacy of 2-AG at cannabinoid receptors. Nat Neurosci 2010;13(8):951–7.
8. Savinainen JR, Saario SM, Laitinen JT. The serine hydrolases MAGL, ABHD6 and ABHD12 as guardians of 2-arachidonoylglycerol signalling through cannabinoid receptors. Acta Physiol 2012;204(2):267–76.
9. Kozak KR, Rowlinson SW, Marnett LJ. Oxygenation of the endocannabinoid, 2-arachidonylglycerol, to glyceryl prostaglandins by cyclooxygenase-2. J Biol Chem 2000;275(43):33744–9.
10. Kozak KR, Crews BC, Morrow JD, Wang L-H, Ma YH, Weinander R, et al. Metabolism of the endocannabinoids, 2-arachidonylglycerol and anandamide, into prostaglandin, thromboxane, and prostacyclin glycerol esters and ethanolamides. J Biol Chem 2002 ;277(47):44877–85.
11. Urquhart P, Nicolaou A, Woodward DF. Endocannabinoids and their oxygenation by cyclo-oxygenases, lipoxygenases and other oxygenases. Biochim Biophys Acta 2015;1851(4):366–76.
12. Long JZ, Nomura DK, Vann RE, Walentiny DM, Booker L, Jin X, et al. Dual blockade of FAAH and MAGL identifies behavioral processes regulated by endocannabinoid crosstalk in vivo. Proc Natl Acad Sci U S A 2009;106(48):20270–5.
13. Pertwee RG. Elevating endocannabinoid levels: pharmacological strategies and potential therapeutic applications. Proc Nutr Soc. 2014;73(1):96–105.
14. McKinney MK, Cravatt BF. Structure and Function of Fatty Acid Amide Hydrolase. Annu Rev Biochem 2005;74(1):411–32.
15. Kathuria S, Gaetani S, Fegley D, Valiño F, Duranti A, Tontini A, et al. Modulation of anxiety through blockade of anandamide hydrolysis. Nat Med. 2003;9(1):76–81.
16. Justinova Z, Mangieri RA, Bortolato M, Chefer SI, Mukhin AG, Clapper JR, et al. Fatty acid amide hydrolase inhibition heightens anandamide signaling without producing reinforcing

effects in primates. Biol Psychiatry 2008;64(11):930–7.

17. Apodaca R, Breitenbucher Jg, Chambers Al, Seierstad M, Xiao W. Oxazolyl piperidine modulators of fatty acid amide hydrolase. WO2007140005 (**2007**).
18. Vacondio F, Silva C, Lodola A, Fioni A, Rivara S, Duranti A, et al. Structure-property relationships of a class of carbamate-based fatty acid amide hydrolase (FAAH) inhibitors: chemical and biological stability. ChemMedChem 2009;4(9):1495–504.
19. Clapper JR, Vacondio F, King AR, Duranti A, Tontini A, Silva C, et al. A second generation of carbamate-based fatty acid amide hydrolase inhibitors with improved activity in vivo. ChemMedChem 2009;4(9):1505–13.
20. Boger DL, , Hiroshi Miyauchi, Wu Du, Christophe Hardouin RA, Fecik, Heng Cheng, Inkyu Hwang, Michael P. Hedrick DL, Orlando Acevedo, Cristiano R. W. Guimarães, William L. Jorgensen and BF, Cravatt. Discovery of a Potent, Selective, and Efficacious Class of Reversible α-Ketoheterocycle Inhibitors of Fatty Acid Amide Hydrolase Effective as Analgesics. 2008;15(10):1203–14.
21. Mauro Mileni, Joie Garfunkle, Jessica K. DeMartino BFC, L. D, Boger and RCS. Binding and Inactivation Mechanism of a Humanized Fatty Acid Amide Hydrolase by α-Ketoheterocycle Inhibitors Revealed from Co-Crystal Structures. 2010;131(30):10497–506.
22. Boger DL. Alpha-keto heterocycles as FAAH inhibitors. WO2010005572 (**2010**)
23. Boger DL. Alpha-ketoheterocycles and methods of making and using. WO2012106569 (**2012**).
24. Cyrine Ezzili, Mauro Mileni, Nicholas McGlinchey JZL, G. S, Kinseyx, , Dustin G. Hochstatter†, Raymond C. Stevens AHL, F. B, Cravatt, Edward J. Bilsky and DLB. Reversible Competitive α-Ketoheterocycle Inhibitors of Fatty Acid Amide Hydrolase Containing Additional Conformational Constraints in the Acyl Side Chain: Orally Active, Long Acting Analgesics. 2008;15(10):1203–14.
25. Mor M, Rivara S, Lodola A, Plazzi PV, Tarzia G, Duranti A, et al. Cyclohexylcarbamic acid 3'- or 4'-substituted biphenyl-3-yl esters as fatty acid amide hydrolase inhibitors: synthesis, quantitative structure-activity relationships, and molecular modeling studies. J Med Chem 2004;47(21):4998–5008.
26. Piomelli D, Duranti A, Tontini A, Mor M, Tarzia G. Modulation of anxiety through blockade of anandamide hydrolysis. WO2004033422 (**2004**).
27. Piomelli D, Tarzia G, Duranti A, Tontini A, Mor M, Compton TR, et al. Pharmacological profile of the selective FAAH inhibitor KDS-4103 (URB597). CNS Drug Rev. 2006;12(1):21–38.
28. Russo R, Loverme J, La Rana G, Compton TR, Parrott J, Duranti A, et al. The fatty acid amide hydrolase inhibitor URB597 (cyclohexylcarbamic acid 3'-carbamoylbiphenyl-3-yl ester) reduces neuropathic pain after oral administration in mice. J Pharmacol Exp Ther. 2007;322(1):236–42.
29. Andrew J. Kwilasz, Rehab A. Abdullah, Justin L. Poklis, Aron H. Lichtman and SS, Negus. Effects of the Fatty Acid Amide Hydrolase (FAAH) Inhibitor URB597 on Pain-Stimulated and Pain-Depressed Behavior in Rats. 2013;18(9):1199–216.

30. Lomazzo E, Bindila L, Remmers F, Lerner R, Schwitter C, Hoheisel U, et al. Therapeutic Potential of Inhibitors of Endocannabinoid Degradation for the Treatment of Stress-Related Hyperalgesia in an Animal Model of Chronic Pain. Neuropsychopharmacology Nature 2015;40(2):488–501.
31. Blankman JL, Cravatt BF. Chemical probes of endocannabinoid metabolism. Pharmacol Rev 2013;65(2):849–71.
32. Piomelli D, Clapper Jr, Moreno-Sanz G, Duranti A, Tontini A, Mor M, et al. Peripherally restricted FAAH inhibitors. WO2012015704 (**2012**).
33. Minetti P, Cabri W, Dallavalle S, Merlini L, Penco S, Borsini F, et al. Enol carbamate derivatives as modulators of fatty acid amide hydrolase. WO2009109504 (**2009**).
34. Minetti P, Cabri W, Borsini F, Caprioli A, Penco S, Dallavalle S, et al. Oxime carbamoyl derivatives as modulators of fatty acid amides hydrolase. WO2009138416 (**2009**).
35. Caprioli A, Coccurello R, Rapino C, Di Serio S, Di Tommaso M, Vertechy M, et al. The novel reversible fatty acid amide hydrolase inhibitor ST4070 increases endocannabinoid brain levels and counteracts neuropathic pain in different animal models. J Pharmacol Exp Ther 2012;342(1):188–95.
36. Cabri W, Minetti P, Campiani G, Butini S. Carbamate Derivatives in particular for the treatment of neurological disorders. WO2010105930 (**2010**).
37. Butini S, Brindisi M, Gemma S, Minetti P, Cabri W, Gallo G, et al. Discovery of potent inhibitors of human and mouse Fatty Acid amide hydrolases. J Med Chem. 2012;55(15):6898–915.
38. Almario-Garcia A, George P, Hoornaert C, Li A-T, Puech F. Alkyl-, alkenyl- et alkynyl carbamate derivatives, the preparation and therapeutic use thereof. WO2006117461 (**2006**).
39. Abouabdellah A, Chereze N, Fayol A, Saady M, Vache J, Veronique C, et al. Carbamate derivatives of alkyl-heterocycles, preparation thereof and therapeutic use thereof. WO2010055267 (**2010**)
40. Abouabdellah A, Görlitzer J, Hamley P, Ravet A. Alkyl thiazole carbamate derivatives, preparation thereof, and use thereof as FAAH enzyme inhibitors. WO2010010288 (**2010**).
41. Abouabdellah A, Fayol A, Lochead A, Saady M, Vache J, Yaiche P. 5-Membered heterocyclic compound cyclopenta[c]pyrrolylalkylcarbamate derivatives, preparation thereof, and therapeutic use thereof. WO2010130943 (**2010**).
42. Abouabdellah A, Even L, Fayol A, Vache J, Yaiche P. Cyclopenta[c]pyrrole-2-carboxylate derivatives, preparation thereof, and therapeutic use thereof. WO2010130944 (**2010**)
43. Abouabdellah A, Chereze N, Fayol A, Lochead A, Saady M, Vache J, et al. 7-Aza-spiro[3.5]nonane-7-carboxylate derivatives, preparation thereof, and therapeutic use thereof. WO2010130945 (**2010**).
44. Abouabdellah A, Chereze N, Fayol A, Lochead A, Saady M, Vache J, et al. Derivatives of azaspiranyl-alkylcarbamates of 5-member heterocyclic compounds, preparation thereof and

therapeutic use thereof. WO2010089510 (**2010**).

45. Abouabdellah A, Almario Garcia A, Hoornaert C, Li At. Derivatives of 1-piperazine- and 1-homopiperazine-carboxylates, preparation method thereof and use of same as inhibitors of the FAAH enzyme. WO2005070910 (**2005**).
46. Johnson AK. Fatty acid amide hydrolase as a potential therapeutic target for the treatment of pain and CNS disorders. Expert Opin Drug Discov. 2009;4(7):763–84.
47. Otrubova K, Ezzili C, Boger DL. The discovery and development of inhibitors of fatty acid amide hydrolase (FAAH). Bioorg Med Chem Lett 2011;21(16):4674–85.
48. Ahn K, Johnson DS, Mileni M, Beidler D, Long JZ, McKinney MK, et al. Discovery and characterization of a highly selective FAAH inhibitor that reduces inflammatory pain. Chem Biol 2009;16(4):411–20.
49. Booker L, Kinsey SG, Abdullah RA, Blankman JL, Long JZ, Ezzili C, et al. The fatty acid amide hydrolase (FAAH) inhibitor PF-3845 acts in the nervous system to reverse LPS-induced tactile allodynia in mice. Br J Pharmacol 2012;165(8):2485–96.
50. Tchantchou F, Tucker LB, Fu AH, Bluett RJ, McCabe JT, Patel S, et al. The fatty acid amide hydrolase inhibitor PF-3845 promotes neuronal survival, attenuates inflammation and improves functional recovery in mice with traumatic brain injury. Neuropharmacology 2014;85:427–39.
51. Johnson DS, Stiff C, Lazerwith SE, Kesten SR, Fay LK, Morris M, et al. Discovery of PF-04457845: A Highly Potent, Orally Bioavailable, and Selective Urea FAAH Inhibitor. ACS Med Chem Lett 2011;2(2):91–6.
52. Ahn K, Smith SE, Liimatta MB, Beidler D, Sadagopan N, Dudley DT, et al. Mechanistic and pharmacological characterization of PF-04457845: a highly potent and selective fatty acid amide hydrolase inhibitor that reduces inflammatory and. J Pharmacol Exp Ther 2011;338(1):114–24.
53. Di Marzo V. Inhibitors of endocannabinoid breakdown for pain: not so FA(AH)cile, after all. Pain 2012 153(9):1785–6.
54. Fay Lk, Johnson Ds, Meyers Mj, Thorarensen A, Wang Lj. Ether benzylidene piperidine aryl carboxamide compounds useful as FAAH inhibitors. WO2009127944 (**2009**).
55. Meyers MJ, Pelc MJ, Thorarensen A. 4- [3- (Aryloxy) benzylidene] -3-methyl piperidine aryl carboxamide compounds useful as FAAH inhibitors. WO2009127949 (**2009**).
56. Long SA, Meyers MJ, Pelc MJ, Schweitzer BA, Thorarensen A, Wang LJ. 7-Azaspiro [3.5] nonane-7-carboxamide compounds as modulators of fatty acid amide hydrolase. WO2010049841 (**2010**).
57. Feledziak M, Lambert DM, Marchand-Brynaert J, Muccioli GG. Inhibitors of the endocannabinoid-degrading enzymes, or how to increase endocannabinoid's activity by preventing their hydrolysis. Recent Pat CNS Drug Discov 2012;7(1):49–70.
58. Roughley S, Walls S, Hart T, Parsons R, Brough P, Graham C, et al. Azetidine derivatives WO2009109743 (**2009**).
59. Breitenbucher J. G, Keith JM, Tichenor MS, Chambers AL, Jones WM, Hawryluk NA, et al.

Heteroaryl-substituted urea modulators of fatty acid amide hydrolase. WO2010068452 (**2010**).

60. Breitenbucher J. G, Keith JM, Tichenor MS, Chambers AL, Jones WM, Hawryluk NA, et al. Heteroaryl-substituted urea modulators of fatty acid amide hydrolase. WO2010068453 (**2010**).
61. Apodaca R, Breitenbucher J. G, Hawryluk NA, Jones WM, Keith JM, Merit JE, et al. Heteroaryl-substituted urea modulators of fatty acid amide hydrolase. WO2008153752 (**2008**).
62. Breitenbucher GJ, Keith JM, Jones WM. Heteroaryl-substituted spirocyclic diamine urea modulators of fatty acid amide hydrolase. WO2010141817 (**2010**).
63. Keith JM, Jones WM, Pierce JM, Seierstad M, Palmer JA, Webb M, et al. Heteroarylureas with spirocyclic diamine cores as inhibitors of fatty acid amide hydrolase. Bioorg Med Chem Lett 2014;24(3):737–41.
64. Lin LS, Chioda MD, Liu P, Nargund RP. Imidazole derivatives useful as inhibitors of FAAH. WO2009152025 (**2009**).
65. Liu P, Devita RJ, Lin LS, Chioda MD, Hamill TG, Li W. Imidazole derivatives useful as modulators of FAAH and as FAAH imaging agents. WO2010101724 (**2010**)
66. Lin LS, Chang LL, Chobanian H, Nargund RP. Pyrazole derivatives useful as inhibitors of FAAH. WO2009151991 (**2009**).
67. Chobanian H, Lin LS, Liu P, Chioda MD, Devita RJ, Nargund RP, et al. Oxazole derivatives useful as inhibitors of FAAH. WO2010017079 (**2010**).
68. Chobanian HR, Guo Y, Liu P, Chioda MD, Fung S, Lanza TJ, et al. Discovery of MK-4409, a Novel Oxazole FAAH Inhibitor for the Treatment of Inflammatory and Neuropathic Pain. ACS Med Chem Lett 2014;5(6):717–21.
69. Boger DL. Oxazole ketones as modulators of fatty acid amide hydrolase. WO2007098142 (**2007**).
70. Banijamali AR, Wakefield JD, Mermerian AH, Busby RW. Metabolism and disposition of MM-433593, a selective FAAH-1 inhibitor, in monkeys. Pharmacol Res Perspect 2014;2(5) e00059
71. Behnke ML, Castro AC, Chan LK, Evans CA, Grenier L, Grogan MJ, et al. Isoxazolines as inhibitors of fatty acid amide hydrolase. WO2010135360 (**2010**).
72. Alapafuja SO, Nikas SP, Bharathan IT, Shukla VG, Nasr ML, Bowman AL, et al. Sulfonyl fluoride inhibitors of fatty acid amide hydrolase. J Med Chem 2012;55(22):10074–89.
73. Panlilio L V., Thorndike EB, Nikas SP, Alapafuja SO, Bandiera T, Cravatt BF, et al. Effects of fatty acid amide hydrolase (FAAH) inhibitors on working memory in rats. Psychopharmacology (Berl) 2015; DOI 10.1007/s00213-015-4140-6.
74. Nicolussi S, Chicca A, Rau M, Rihs S, Soeberdt M, Abels C, et al. Correlating FAAH and anandamide cellular uptake inhibition using N-alkylcarbamate inhibitors: from ultrapotent to hyperpotent. Biochem Pharmacol 2014;92(4):669–89.
75. Labar G, Bauvois C, Borel F, Ferrer JL, Wouters J, Lambert DM. Crystal structure of the human monoacylglycerol lipase, a key actor in endocannabinoid signaling. ChemBioChem. 2010;11(2):218–27.

76. King a R, Lodola a, Carmi C, Fu J, Mor M, Piomelli D. A critical cysteine residue in monoacylglycerol lipase is targeted by a new class of isothiazolinone-based enzyme inhibitors. Br J Pharmacol 2009;157(6):974–83.
77. Saario SM, Salo OMH, Nevalainen T, Poso A, Laitinen JT, Järvinen T, et al. Characterization of the sulfhydryl-sensitive site in the enzyme responsible for hydrolysis of 2-arachidonoyl-glycerol in rat cerebellar membranes. Chem Biol. 2005;12(6):649–56.
78. Long JZ, Nomura DK, Cravatt BF. Characterization of monoacylglycerol lipase inhibition reveals differences in central and peripheral endocannabinoid metabolism. Chem Biol 2009;16(7):744–53.
79. Muccioli GG, Labar G, Lambert DM. CAY10499, A Novel Monoglyceride Lipase Inhibitor Evidenced by an Expeditious MGL Assay. ChemBioChem 2008;9(16):2704–10.
80. Chang JW, Niphakis MJ, Lum KM, Cognetta AB, Wang C, Matthews ML, et al. Highly selective inhibitors of monoacylglycerol lipase bearing a reactive group that is bioisosteric with endocannabinoid substrates. Chem Biol 2012;19(5):579–88.
81. Niphakis MJ, Cognetta AB, Chang JW, Buczynski MW, Parsons LH, Byrne F, et al. Evaluation of NHS carbamates as a potent and selective class of endocannabinoid hydrolase inhibitors. ACS Chem Neurosci 2013;4(9):1322–32.
82. Griebel G, Pichat P, Beeské S, Leroy T, Redon N, Jacquet A, et al. Selective blockade of the hydrolysis of the endocannabinoid 2-arachidonoylglycerol impairs learning and memory performance while producing antinociceptive activity in rodents. Sci Rep 2015;5:7642.
83. Bisogno T, Ortar G, Petrosino S, Morera E, Palazzo E, Nalli M, et al. Development of a potent inhibitor of 2-arachidonoylglycerol hydrolysis with antinociceptive activity in vivo. Biochim Biophys Acta 2009;1791(1):53–60.
84. Makriyannis A, Nikas, Spyridon P, Alapafuja, Shakiru O, Shukla, Vidyanand G. Monoacylglycerol lipase inhibitors for modulation of cannabinoid activity. WO2009052319 (**2009**).
85. Zvonok N, Pandarinathan L, Williams J, Johnston M, Karageorgos I, Janero DR, et al. Covalent inhibitors of human monoacylglycerol lipase: ligand-assisted characterization of the catalytic site by mass spectrometry and mutational analysis. Chem Biol 2008;15(8):854–62.
86. Bertrand T, Augé F, Houtmann J, Rak A, Vallée F, Mikol V, et al. Structural basis for human monoglyceride lipase inhibition. J Mol Biol 2010; 396(3):663–73.
87. Even L, Hoornaert C. Triazolopyridine carboxamide derivatives and triazolopyrimidine carboxamide derivatives, preparation thereof and therapeutic use thereof. WO2008145839 (**2008**).
88. Hoornaert C. Triazolopyridine carboxamide derivatives, preparation thereof and therapeutic use thereof. WO2008145842 (**2008**).
89. Even L. Triazolopyridine carboxamide derivatives and triazolopyrimidine carboxamide derivatives, preparation thereof and therapeutic. WO2008145839 (**2008**).

90. Morera L, Labar G, Ortar G, Lambert DM. Development and characterization of endocannabinoid hydrolases FAAH and MAGL inhibitors bearing a benzotriazol-1-yl carboxamide scaffold. Bioorg Med Chem 2012;20(21):6260–75.
91. Aaltonen N, Savinainen JR, Ribas CR, Rönkkö J, Kuusisto A, Korhonen J, et al. Piperazine and piperidine triazole ureas as ultrapotent and highly selective inhibitors of monoacylglycerol lipase. Chem Biol 2013; 20(3):379–90.
92. Chicca A, Marazzi J, Gertsch J. The antinociceptive triterpene β-amyrin inhibits 2-arachidonoylglycerol (2-AG) hydrolysis without directly targeting cannabinoid receptors. Br J Pharmacol 2012;167(8):1596–608.
93. King AR, Dotsey EY, Lodola A, Jung KM, Ghomian A, Qiu Y, et al. Discovery of potent and reversible monoacylglycerol lipase inhibitors. Chem Biol 2009;16(10):1045–52.
94. Hernández-Torres G, Cipriano M, Hedén E, Björklund E, Canales Á, Zian D, et al. A Reversible and Selective Inhibitor of Monoacylglycerol Lipase Ameliorates Multiple Sclerosis. Angew Chemie Int Ed 2014; 53(50):13765–70.
95. Long JZ, Li W, Booker L, Burston JJ, Kinsey SG, Schlosburg JE, et al. Selective blockade of 2-arachidonoylglycerol hydrolysis produces cannabinoid behavioral effects. Nat Chem Biol 2009;5(1):37–44.
96. Kinsey SG, Long JZ, O'Neal ST, Abdullah RA, Poklis JL, Boger DL, et al. Blockade of endocannabinoid-degrading enzymes attenuates neuropathic pain. J Pharmacol Exp Ther 2009;330(3):902–10.
97. Ghosh S, Wise LE, Chen Y, Gujjar R, Mahadevan A, Cravatt BF, et al. The monoacylglycerol lipase inhibitor JZL184 suppresses inflammatory pain in the mouse carrageenan model. Life Sci 2013; 92(8-9):498–505.
98. Kinsey SG, O'Neal ST, Long JZ, Cravatt BF, Lichtman AH. Inhibition of endocannabinoid catabolic enzymes elicits anxiolytic-like effects in the marble burying assay. Pharmacol Biochem Behav 2011;98(1):21–7.
99. Ignatowska-Jankowska BM, Ghosh S, Crowe MS, Kinsey SG, Niphakis MJ, Abdullah RA, et al. *In vivo* characterization of the highly selective monoacylglycerol lipase inhibitor KML29: antinociceptive activity without cannabimimetic side effects. Br J Pharmacol 2014;171(6):1392–407.
100. Pasquarelli N, Porazik C, Hanselmann J, Weydt P, Ferger B, Witting A. Comparative biochemical characterization of the monoacylglycerol lipase inhibitor KML29 in brain, spinal cord, liver, spleen, fat and muscle tissue. Neuropharmacology 2015;91:148–56.
101. Chen R, Zhang J, Wu Y, Wang D, Feng G, Tang Y-P, et al. Monoacylglycerol lipase is a therapeutic target for Alzheimer's disease. Cell Rep 2012; 2(5):1329–39.
102. Pan B, Wang W, Zhong P, Blankman JL, Cravatt BF, Liu Q -s. Alterations of Endocannabinoid Signaling, Synaptic Plasticity, Learning, and Memory in Monoacylglycerol Lipase Knock-out Mice. J Neurosci 2011;31(38):13420–30.

103. Lysenko L V, Kim J, Henry C, Tyrtyshnaia A, Kohnz RA, Madamba F, et al. Monoacylglycerol lipase inhibitor JZL184 improves behavior and neural properties in Ts65Dn mice, a model of down syndrome. PLoS One2014;9(12):e114521.
104. Schlosburg JE, Blankman JL, Long JZ, Nomura DK, Pan B, Kinsey SG, et al. Chronic monoacylglycerol lipase blockade causes functional antagonism of the endocannabinoid system. Nat Neurosci Nature Publishing Group; 2010;13(9):1113–9.
105. Kinsey SG, Wise LE, Ramesh D, Abdullah RA, Selley DE, Cravatt BF, et al. Repeated low-dose administration of the monoacylglycerol lipase inhibitor JZL184 retains cannabinoid receptor type 1-mediated antinociceptive and gastroprotective effects. J Pharmacol Exp Ther 2013;345(3):492–501.
106. Chanda PK, Gao Y, Mark L, Btesh J, Strassle BW, Lu P, et al. Monoacylglycerol Lipase Activity Is a Critical Modulator of the Tone and Integrity of the Endocannabinoid System 2010;78(6):996–1003.
107. Sciolino NR, Zhou W, Hohmann AG. Enhancement of endocannabinoid signaling with JZL184, an inhibitor of the 2-arachidonoylglycerol hydrolyzing enzyme monoacylglycerol lipase, produces anxiolytic effects under conditions of high environmental aversiveness in rats. Pharmacol Res2011;64(3):226–34.
108. Busquets-Garcia A, Puighermanal E, Pastor A, de la Torre R, Maldonado R, Ozaita A. Differential role of anandamide and 2-arachidonoylglycerol in memory and anxiety-like responses. Biol Psychiatry 2011;70(5):479–86.
109. Crowe MS, Nass SR, Gabella KM, Kinsey SG. The endocannabinoid system modulates stress, emotionality, and inflammation. Brain Behav Immun 2014; 42:1–5.
110. von Rüden EL, Bogdanovic RM, Wotjak CT, Potschka H. Inhibition of monoacylglycerol lipase mediates a cannabinoid 1-receptor dependent delay of kindling progression in mice. Neurobiol Dis 2015;77:238–45.
111. Zhong P, Wang W, Pan B, Liu X, Zhang Z, Long JZ, et al. Monoacylglycerol Lipase Inhibition Blocks Chronic Stress-Induced Depressive-Like Behaviors via Activation of mTOR Signaling. Neuropsychopharmacology 2014; 39(7):1763–76.
112. Sticht MA, Long JZ, Rock EM, Limebeer CL, Mechoulam R, Cravatt BF, et al. Inhibition of monoacylglycerol lipase attenuates vomiting in Suncus murinus and 2-arachidonoyl glycerol attenuates nausea in rats. Br J Pharmacol 2012;165(8):2425–35.
113. Schlosburg JE, Blankman JL, Long JZ, Nomura DK, Pan B, Kinsey SG, et al. Chronic monoacylglycerol lipase blockade causes functional antagonism of the endocannabinoid system. 2010;13(9).
114. Mangieri RA, Piomelli D. Enhancement of endocannabinoid signaling and the pharmacotherapy of depression. Pharmacol Res 2007;56(5):360–6.
115. Marrs WR, Horne E a., Ortega-Gutierrez S, Cisneros JA, Xu C, Lin YH, et al. Dual inhibition of α/β-hydrolase domain 6 and fatty acid amide hydrolase increases endocannabinoid levels in

neurons. J Biol Chem. 2011;286(33):28723–8.
116. Emma L Scotter1 MEA and MG. The endocannabinoid system as a target for the treatment of motor dysfunction. Br J Pharmacol. 2009;156(7):1029–40.
117. Li W, Blankman JL, Cravatt BF. A functional proteomic strategy to discover inhibitors for uncharacterized hydrolases. J Am Chem Soc 2007;129(31):9594–5.
118. Navia-Paldanius D, Savinainen JR, Laitinen JT. Biochemical and pharmacological characterization of human α/β-hydrolase domain containing 6 (ABHD6) and 12 (ABHD12). J Lipid Res. 2012;53(11):2413–24.
119. Bachovchin DA, Ji T, Li W, Simon GM, Blankman JL, Adibekian A, et al. Superfamily-wide portrait of serine hydrolase inhibition achieved by library-versus-library screening. Proc Natl Acad Sci U S A 2010;107(49):20941–6.
120. Hsu K-L, Tsuboi K, Adibekian A, Pugh H, Masuda K, Cravatt BF. DAGLβ inhibition perturbs a lipid network involved in macrophage inflammatory responses. Nat Chem Biol 2012;8(12):999–1007.
121. Hsu KL, Tsuboi K, Chang JW, Whitby LR, Speers AE, Pugh H, et al. Discovery and Optimization of Piperidyl-1, 2, 3-Triazole Ureas as Potent, Selective, and in Vivo-Active Inhibitors of α/β-Hydrolase Domain Containing 6 (ABHD6). J Med Chem. 2013;56(21):8270–9.
122. Tchantchou F, Zhang Y. Selective inhibition of alpha/beta-hydrolase domain 6 attenuates neurodegeneration, alleviates blood brain barrier breakdown, and improves functional recovery in a mouse model of traumatic brain injury. J Neurotrauma 2013;30(7):565–79.
123. Wen J, Ribeiro R, Tanaka M, Zhang Y. Activation of CB2 receptor is required for the therapeutic effect of ABHD6 inhibition in experimental autoimmune encephalomyelitis. Neuropharmacology 2015;99:196–209.
124. Naydenov A V., Horne E a., Cheah CS, Swinney K, Hsu KL, Cao JK, et al. ABHD6 blockade exerts antiepileptic activity in PTZ-induced seizures and in spontaneous seizures in R6/2 Mice. Neuron. 2014;83(2):361–71.
125. Jain KK. Neuroprotection in traumatic brain injury. Drug Discov Today 2008;13(23-24):1082–9.
126. Loane DJ, Faden AI. Neuroprotection for traumatic brain injury: translational challenges and emerging therapeutic strategies. Trends Pharmacol Sci 2010;31(12):596–604.
127. Lucchinetti C, Brück W, Parisi J, Scheithauer B, Rodriguez M, Lassmann H. Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. Ann Neurol 2000;47(6):707–17.
128. Engelhardt B. Regulation of immune cell entry into the central nervous system. Results Probl Cell Differ 2006;43:259–80.
129. Noseworthy JH, Lucchinetti C, Rodriguez M, Weinshenker BG. Multiple sclerosis. N Engl J Med 2000;343(13):938–52.
130. Gimferrer I, Calvo M, Mittelbrunn M, Farnós M, Sarrias MR, Enrich C, et al. Relevance of CD6-mediated interactions in T cell activation and proliferation. J Immunol 2004;173(4):2262–

70.

131. Sigel E, Baur R, Racz I, Marazzi J, Smart TG, Zimmer a., et al. The major central endocannabinoid directly acts at GABAA receptors. Proc Natl Acad Sci. 2011;108(44):18150–5.
132. Baur R, Kielar M, Richter L, Ernst M, Ecker GF, Sigel E. Molecular analysis of the site for 2-arachidonylglycerol (2-AG) on the β_2 subunit of GABA(A) receptors. J Neurochem2013 Jul;126(1):29–36.
133. Fiskerstrand T, Brahim DH, Johansson S, Abderrahim M, Haukanes BI, Drouot N, et al. Mutations in ABHD12 Cause the Neurodegenerative Disease PHARC : An Inborn Error of Endocannabinoid Metabolism. Am J Hum Genet 2010;87(3):410–7.
134. Fiskerstrand T, Knappskog P, Majewski J, Wanders RJ, Boman H, Bindoff L a. A novel Refsum-like disorder that maps to chromosome 20. Neurology 2009;72(1):20–7.
135. Blankman JL, Long JZ, Trauger SA, Siuzdak G, Cravatt BF. ABHD12 controls brain lysophosphatidylserine pathways that are deregulated in a murine model of the neurodegenerative disease PHARC. 2013;110(4):1–6.
136. Parkkari T, Haavikko R, Laitinen T, Navia-Paldanius D, Rytilahti R, Vaara M, et al. Discovery of triterpenoids as reversible inhibitors of α/β-hydrolase domain containing 12 (ABHD12). PLoS One 2014;9(5):e98286.
137. Nicolussi S, Gertsch J. Endocannabinoid transport revisited. Vitam Horm 2015;98:441–85.
138. Chicca A, Marazzi J, Nicolussi S, Gertsch J. Evidence for bidirectional endocannabinoid transport across cell membranes. J Biol Chem. 2012;287(41):34660–82.
139. Burch P, Chicca A, Gademann K. Study and Investigations on Their Mode of Action. 2014;1–6.
140. Ghilardi JR. Constitutive Spinal Cyclooxygenase-2 Participates in the Initiation of Tissue Injury-Induced Hyperalgesia. J Neurosci 2004;24(11):2727–32.
141. Nomura DK, Lombardi DP, Chang JW, Niessen S, Ward AM, Long JZ, et al. Monoacylglycerol lipase exerts dual control over endocannabinoid and fatty acid pathways to support prostate cancer. Chem Biol 2011;18(7):846–56.
142. Kozak KR, Crews BC, Ray JL, Tai HH, Morrow JD, Marnett LJ. Metabolism of Prostaglandin Glycerol Esters and Prostaglandin Ethanolamides in Vitro and in Vivo. J Biol Chem. 2001;276(40):36993–8.
143. Kozak KR, Prusakiewicz JJ, Rowlinson SW, Marnett LJ. Enantiospecific, selective cyclooxygenase-2 inhibitors. Bioorg Med Chem Lett 2002;12(9):1315–8.
144. Rouzer CA, Marnett LJ. Endocannabinoid Oxygenation by Cyclooxygenases, Lipoxygenases, and Cytochromes P450: Cross-Talk between the Eicosanoid and Endocannabinoid Signaling Pathways. Chem Rev 2011;111(10):5899–921.
145. Liang Y, Woodward DF, Guzman VM, Li C, Scott DF, Wang JW, et al. Identification and pharmacological characterization of the prostaglandin FP receptor and FP receptor variant complexes. Br J Pharmacol. 2008;154(5):1079–93.
146. Woodward DF, Krauss a H, Wang JW, Protzman CE, Nieves a L, Liang Y, et al. Identification

of an antagonist that selectively blocks the activity of prostamides (prostaglandin-ethanolamides) in the feline iris. Br J Pharmacol 2007;150(3):342–52.

147. Woodward DF, Liang Y, Krauss AH-P. Prostamides (prostaglandin-ethanolamides) and their pharmacology. Br J Pharmacol 2008;153(3):410–9.
148. Jones RL, Giembycz M a., Woodward DF. Prostanoid receptor antagonists: Development strategies and therapeutic applications. Br J Pharmacol. 2009;158(April):104–45.
149. Gatta L, Piscitelli F, Giordano C, Boccella S, Lichtman A, Maione S, et al. Discovery of prostamide F2α and its role in inflammatory pain and dorsal horn nociceptive neuron hyperexcitability. PLoS One 2012;7(2):e31111.
150. Ligresti A, Martos J, Wang J, Guida F, Allarà M, Palmieri V, et al. Prostamide F2α receptor antagonism combined with inhibition of FAAH may block the pro-inflammatory mediators formed following selective FAAH inhibition. Br J Pharmacol. 2014;171(6):1408–19.
151. Dong L, Vecchio AJ, Sharma NP, Jurban BJ, Malkowski MG, Smith WL. Human cyclooxygenase-2 is a sequence homodimer that functions as a conformational heterodimer. J Biol Chem. 2011;286(21):19035–46.
152. Duggan KC, Hermanson DJ, Musee J, Prusakiewicz JJ, Scheib JL, Carter BD, et al. (R)-Profens are substrate-selective inhibitors of endocannabinoid oxygenation by COX-2. Nat Chem Biol Nature Publishing Group; 2011;7(11):803–9.
153. Hermanson DJ, Hartley ND, Gamble-George J, Brown N, Shonesy BC, Kingsley PJ, et al. Substrate-selective COX-2 inhibition decreases anxiety via endocannabinoid activation. Nat Neurosci 2013;16(9):1291–8.
154. Alhouayek M, Masquelier J, Cani PD, Lambert DM, Muccioli GG. Implication of the anti-inflammatory bioactive lipid prostaglandin D2-glycerol ester in the control of macrophage activation and inflammation by ABHD6. Proc Natl Acad Sci U S A 2013;110(43):17558–63.
155. Alhouayek M, Muccioli GG. COX-2-derived endocannabinoid metabolites as novel inflammatory mediators. Trends Pharmacol Sci 2014;35(6):284–92.
156. Chicca A, Caprioglio D, Minassi A, Petrucci V, Appendino G, Taglialatela-Scafati O, et al. Functionalization of β-caryophyllene generates novel polypharmacology in the endocannabinoid system. ACS Chem Biol. 2014;9:1499–507.
157. Cipriano M, Björklund E, Wilson AA, Congiu C, Onnis V, Fowler CJ. Inhibition of fatty acid amide hydrolase and cyclooxygenase by the N-(3-methylpyridin-2-yl)amide derivatives of flurbiprofen and naproxen. Eur J Pharmacol 2013;720(1-3):383–90.
158. Grim TW, Ghosh S, Hsu K-L, Cravatt BF, Kinsey SG, Lichtman AH. Combined inhibition of FAAH and COX produces enhanced anti-allodynic effects in mouse neuropathic and inflammatory pain models. Pharmacol Biochem Behav 2014 Sep;124:405–11.
159. Holt S, Paylor B, Boldrup L, Alajakku K, Vandevoorde S, Sundström A, et al. Inhibition of fatty acid amide hydrolase, a key endocannabinoid metabolizing enzyme, by analogues of ibuprofen and indomethacin. Eur J Pharmacol. 2007;565(1-3):26–36.

160. Fowler CJ, Björklund E, Lichtman AH, Naidu PS, Congiu C, Onnis V. Inhibitory properties of ibuprofen and its amide analogues towards the hydrolysis and cyclooxygenation of the endocannabinoid anandamide. J Enzyme Inhib Med Chem. 2012;(November 2011):1–11.
161. Favia AD, Habrant D, Scarpelli R, Migliore M, Albani C, Bertozzi SM, et al. Identification and characterization of carprofen as a multitarget fatty acid amide hydrolase/cyclooxygenase inhibitor. J Med Chem [2012;55(20):8807–26.
162. Gouveia-Figueira S, Karlsson J, Deplano A, Hashemian S, Svensson M, Fredriksson Sundbom M, et al. Characterisation of (R)-2-(2-Fluorobiphenyl-4-yl)-N-(3-Methylpyridin-2-yl)Propanamide as a Dual Fatty Acid Amide Hydrolase: Cyclooxygenase Inhibitor. PLoS One 2015;10(9):e0139212.
163. Crowe MS, Leishman E, Banks ML, Gujjar R, Mahadevan A, Bradshaw HB, et al. Combined inhibition of monoacylglycerol lipase and cyclooxygenases synergistically reduces neuropathic pain in mice. Br J Pharmacol. 2015;172(7):1700–12.
164. Schuehly W, Paredes JMV, Kleyer J, Huefner A, Anavi-Goffer S, Raduner S, et al. Mechanisms of osteoclastogenesis inhibition by a novel class of biphenyl-type cannabinoid CB(2) receptor inverse agonists. Chem Biol 2011;18(8):1053–64.
165. Chicca A, Gachet MS, Petrucci V, Schuehly W, Charles R-P, Gertsch J. 4′-O-methylhonokiol increases levels of 2-arachidonoyl glycerol in mouse brain via selective inhibition of its COX-2-mediated oxygenation. J Neuroinflammation 2015;12(1):89.
166. Rempel V, Fuchs A, Hinz S, Karcz T, Lehr M, Koetter U, et al. Magnolia Extract, Magnolol, and Metabolites: Activation of Cannabinoid CB2 Receptors and Blockade of the Related GPR55. ACS Med Chem Lett 2012;4(1):41–5.
167. Kim J, Alger BE. Inhibition of cyclooxygenase-2 potentiates retrograde endocannabinoid effects in hippocampus. Nat Neurosci 2004;7(7):697–8.
168. Straiker A, Wager-Miller J, Hu SS, Blankman JL, Cravatt BF, Mackie K. COX-2 and fatty acid amide hydrolase can regulate the time course of depolarization-induced suppression of excitation. Br J Pharmacol 2011;164(6):1672–83.
169. Han H, Jung JK, Han SB, Nam SY, Oh KW, Hong JT. Anxiolytic-Like Effects of 4-O-Methylhonokiol Isolated from Magnolia officinalis Through Enhancement of GABAergic Transmission and Chloride Influx. J Med Food 2011;14(7-8):724–31.
170. Lee Y-J, Choi D-Y, Choi IS, Kim KH, Kim YH, Kim HM, et al. Inhibitory effect of 4-O-methylhonokiol on lipopolysaccharide-induced neuroinflammation, amyloidogenesis and memory impairment via inhibition of nuclear factor-kappaB in vitro and in vivo models. J Neuroinflammation BioMed Central Ltd; 2012;9(1):35.
171. Jung YY, Lee YJ, Choi DY, Hong JT. Amelioration of Cognitive Dysfunction in APP/PS1 Double Transgenic Mice by Long-Term Treatment of 4-O-Methylhonokiol. Biomol Ther (Seoul) 2014;22(3):232–8.

Figure Legends

Figure 1. Development stage for the inhibitors hitting different targets of the endocannabinoid system. Inhibitors for ABHD-6, COX-2 (substrate specific) and EMT already showed some beneficial effects in different animal models. Nonetheless further investigations are needed to generate more drug-like compounds and to identify suitable indications to be pursued in clinical development. For ABHD-12 selective inhibitors are still missing.

Figure 2. Chemical structures of α-ketoheterocycle-based FAAH inhibitor

Figure 3. Chemical structures of carbamate-based FAAH inhibitor

Figure 4. Urea-type FAAH inhibitors

Figure 5. Miscellaneous FAAH inhibitors

Figure 6. Chemical structures of MAGL inhibitors

Figure 7. Miscellaneous MAGL inhibitors

Figure 8. Selective and nonselective ABHDs inhibitors

Figure 9. Endocannabinoid transporter inhibitors

Figure 10. COX-2 substrate specific inhibitors and $PGF_{2\alpha}$-receptor antagonists

Figure 11. Inhibitors targeting 2 or more components of the ECS

Figure 12. Targeting the endocannabinoid system beyond the cannabinoid receptors