Chem Soc Rev

REVIEW ARTICLE

View Article Online
View Journal

Cite this: DOI: 10.1039/c4cs00047a

Chemical approaches to therapeutically target the metabolism and signaling of the endocannabinoid 2-AG and eicosanoids

Rebecca A. Kohnz and Daniel K. Nomura*

Received 27th January 2014

DOI: 10.1039/c4cs00047a

www.rsc.org/csr

The endocannabinoid system, most popularly known as the target of the psychoactive component of marijuana, Δ^9-tetrahydrocannabinol (THC), is a signaling network that modulates a diverse range of physiological processes including nociception, behavior, cognitive function, appetite, metabolism, motor control, memory formation, and inflammation. While THC and its derivatives have garnered notoriety in the eyes of the public, the endocannabinoid system consists of two endogenous signaling lipids, 2-arachidonoylglycerol (2-AG) and *N*-arachidonoylethanolamine (anandamide), which activate cannabinoid receptors CB1 and CB2 in the nervous system and peripheral tissues. This review will focus on the recent efforts to chemically manipulate 2-AG signaling through the development of inhibitors of the 2-AG-synthesizing enzyme diacylglycerol lipase (DAGL) or the 2-AG-degrading enzyme monoacylglycerol lipase (MAGL), and assessing the therapeutic potential of DAGL and MAGL inhibitors in pain, inflammation, degenerative diseases, tissue injury, and cancer.

1. Introduction

The endocannabinoid system is a neurotransmission pathway and the primary target of the psychoactive ligand in marijuana, Δ^9-tetrahydrocannabinol (THC). Marijuana has been in use for centuries for both medicinal and recreational purposes and has profound effects on nociception, behavior, cognitive function, appetite, metabolism, motor control, memory formation, and immune suppression.[1,2] While THC has gained a certain notoriety in the public eye, the physiological function of the endocannabinoid system is to respond to endogenous signaling lipids, 2-arachidonoylglycerol (2-AG) and *N*-arachidonoylethanolamine (anandamide).[3,4] THC and endocannabinoids act through the membrane-bound G-coupled protein receptors cannabinoid receptors type 1 and 2 (CB1 and CB2) to alter these varied aspects of mammalian physiology. CB1 is highly expressed in the central nervous system and to a lower extent in peripheral tissues. CB1 activation appears to control most of the neurogenic features associated with cannabinoid exposure, including hypothermia, hypomotility, anti-nociception, and catalepsy. In contrast, CB2 is expressed predominantly in immune cells such as monocytes, macrophages, CD4+ and CD8+ T cells, and B cells. Originally described as a peripheral cannabinoid receptor, mounting evidence shows that CB2 is also expressed in microglia, which are derived from macrophages, during neuroinflammatory and neurodegenerative disease states.[4–10]

In recent decades, innovative chemical approaches and proteomic and metabolomic technologies have been applied to the endocannabinoid field towards understanding the roles of endocannabinoid signaling lipids in physiology and disease, through the development of inhibitors for endocannabinoid synthesis or degradation. 2-AG is synthesized by diacylglycerol lipase (DAGL) and is degraded by monoacylglycerol lipase (MAGL). Anandamide is synthesized by initial generation of *N*-arachidonoyl phosphatidylethanolamine followed by several postulated routes, and degraded by fatty acid amide hydrolase (FAAH). The (patho)physiological roles, biochemical regulation, and therapeutic potential of FAAH, FAAH inhibitors, and anandamide have been previously studied and reviewed extensively.[4,11] In this review, we will instead focus on chemical approaches that have been applied to understand 2-AG signaling and metabolism and its (patho)physiological roles in various disease states. We will also discuss the therapeutic potential of inhibitors for 2-AG degradation and synthesis.

2. Endocannabinoid signaling

Endocannabinoid signaling in neurons occurs by a non-vesicular calcium-dependent retrograde mechanism. Stimulation of the post-synapse triggers synthesis of endocannabinoids and their subsequent release, although the mechanism by which the

Program in Metabolic Biology, University of California, Berkeley, 127 Morgan Hall, Berkeley, CA 94720, USA. E-mail: dnomura@berkeley.edu

endocannabinoid ligand travels to CB1 receptors at the presynaptic interneuron terminal is poorly understood.[5] CB1 activation inhibits neurotransmitter release by activating $G_{i/o}$ proteins, thereby inhibiting calcium and potassium channels.[12] Originally discovered in 1995 as the second endocannabinoid signaling lipid, 2-AG has been shown to be the major mediator of CB1-dependent synaptic plasticity controlling retrograde neurotransmission through depolarization-induced suppression of inhibition (DSI) and excitation (DSE).[13–19] Endocannabinoids are lipid messengers rather than water-soluble metabolites; thus hydrophobic interactions make their storage in synaptic vesicles unlikely. Instead, endocannabinoids are likely mobilized "on demand" from membrane phospholipid precursors or potential storage sites such as lipid rafts.[5,20]

3. Generation of inhibitors for 2-AG degradation and synthesis

Understanding the physiological roles of 2-AG signaling has been greatly accelerated in recent years through the development of enzymatic inhibitors for 2-AG metabolism.

3.1 Enzymes controlling 2-AG degradation and synthesis

3.1.1 Monoacylglycerol lipase (MAGL). 2-AG is degraded primarily by monoacylglycerol lipase (MAGL) to glycerol and arachidonic acid both *in vitro* and *in vivo* (Fig. 1).[4,11,21] MAGL is a soluble serine hydrolase that peripherally associates with cell membranes and was originally isolated from adipose tissues as the enzyme responsible for the final lipolytic step in triacylglycerol catabolism. Immunodepletion of MAGL in rat brain reduced 2-AG hydrolytic activity by 50%.[22–25] Functional proteomic profiling of 2-AG hydrolytic activity *in vitro* showed that MAGL in the brain is responsible for 85% of total 2-AG hydrolytic activity.[26] MAGL-deficient mice show dramatically elevated levels of 2-AG levels in brain and peripheral tissues.[27] Interestingly, these mice show partial desensitization of CB1 in the brain and blunted responses to exogenous CB1 agonists due to functional antagonism of the endocannabinoid system.[27] Pan *et al.* showed that MAGL −/− mice selectively enhanced theta burst stimulation-induced long-term potentiation in the CA1 region of hippocampal slices.[13,16]

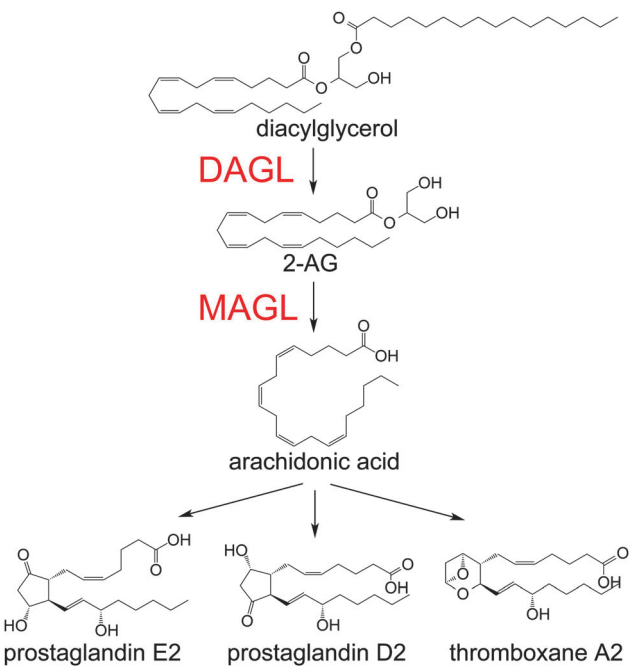

Fig. 1 Pathways that control 2-AG degradation and synthesis. DAGL synthesizes 2-AG through hydrolysis of diacylglycerols and MAGL generates arachidonic acid for eicosanoid biosynthesis through the hydrolysis of 2-AG.

There are also other serine hydrolases that have been implicated in 2-AG hydrolysis. Previous studies using inhibitors of MAGL in mice have found that approximately 15% of 2-AG hydrolytic activity persists after MAGL inhibition. Blankman *et al.* established that the serine hydrolases, α/β-hydrolase 6 and 12 (ABHD6 and 12), were responsible for the remaining 2-AG hydrolytic activity.[26] While it is unclear what role ABHD6 and ABHD12 may play in 2-AG metabolism and signaling, recent studies indicate that these enzymes may have alternate physiological functions. Thomas *et al.* recently showed that genetic knockdown of ABHD6 protects mice against diet-induced obesity and acts as a general lysophospholipid hydrolase that turns over lysophosphatidylglycerol, lysophosphatidylethanolamine, lysophosphatidic acid, and lysophosphatidylserine.[28] Blankman *et al.* recently discovered that ABHD12 hydrolyzes lysophosphatidylserine (LPS) and that ABHD12-deficient mice have elevated levels of brain LPS lipids, but not 2-AG, leading to increased Toll-like receptor activation and age-dependent microglial activation and auditory and motor deficits that resemble the behavioral phenotypes of human polyneuropathy, hearing loss, ataxia, retinosis, and cataract (PHARC) disorder caused by ABHD12 loss-of-function.[29]

3.1.2 Diacylglycerol lipases (DAGL). The biosynthetic pathway for 2-AG relies mainly on two enzymes, diacylglycerol lipase-α and -β (DAGLα and DAGLβ), to synthesize 2-AG from hydrolysis of arachidonoyl-containing diacylglycerols (DAGs) (Fig. 1). DAGs are thought to be synthesized from membrane-bound phospholipids, primarily from *sn*-2 arachidonoyl phosphatidylinositol 4,5-bisphosphate by phospholipase C_β. Two independent studies have confirmed the importance of the two DAGL isoforms in generating 2-AG *in vivo*. Interestingly, these studies have also demonstrated differential contributions of these two isoforms to 2-AG synthesis across various tissues. DAGLα primarily regulates 2-AG levels in the brain, with DAGLα and DAGLβ knockout mice showing ~80% and 50% reduction in brain 2-AG levels, respectively.[17,19] Interestingly, DAGLα knockout mice show a dramatic reduction of 2-AG in the cortex, cerebellum, hypothalamus, and hippocampus while DAGLβ knockout mice showed lower 2-AG levels only in the hypothalamus, indicating differential contributions of these two isoforms even within different regions in the brain.[17,19] In contrast to the brain, DAGLβ is the dominant enzyme for 2-AG synthesis in the liver as evidenced by a ~90% reduction in liver 2-AG levels in DAGLβ knockout mice, compared to ~50–60% reduction in 2-AG levels in DAGLα-deficient livers. Studies using these knockout mice have

shown an important role for the two isoforms of DAGL in retrograde endocannabinoid signaling and adult neurogenesis. The transient suppression of GABA-mediated transmission at inhibitory synapses induced by post-synaptic release of endocannabinoids is lost in DAGLα knockout mice, but not in DAGLβ knockout mice. Both DAGLα and DAGLβ knockout mice show compromised control of adult neurogenesis in the hippocampus or subventricular zone. These studies thus show that DAGL activity in the brain is essential for regulating retrograde synaptic plasticity and adult neurogenesis.[17,19]

3.2 First-generation MAGL and DAGL inhibitors

3.2.1 First-generation MAGL inhibitors. First-generation MAGL inhibitors were non-selective or had modest *in vivo* activity (Fig. 2a). Nonetheless, these inhibitors were initially used to indicate that MAGL was a 2-AG hydrolase and that MAGL blockade led to increased brain 2-AG levels in mice and rats. Both MAGL and FAAH activities can be attenuated with general serine hydrolase inhibitors such as methyl arachidonoyl-fluorophosphonate, phenylmethanesulfonyl fluoride, arachidonoyl trifluoromethylketone, and hexadecyl sulfonyl fluoride.[22,30] MAGL, unlike FAAH and other serine hydrolases, is also sensitive to sulfydryl-specific inhibitors such as mercury chloride, 4-chloro-mercuribenzoic acid, and *N*-ethylmaleimide, which is indicative of a free cysteine residue near the active site. The first semi-selective MAGL inhibitors URB602, *N*-arachidonoyl maleimide (NAM), and OMDM169 exhibited modest increase in 2-AG concentration and proved to be effective against rodent models of pain. The carbamate compound URB602 showed an approximately two-fold increase in the concentration of 2-AG, but not anandamide, in rat central gray matter.[31] URB602 has low potency *in vivo* and possible overlapping selectivity with FAAH *in vitro*,[31–33] making it unsuitable for work distinguishing the functions of these two enzymes. NAM was found to nearly abolish 2-AG hydrolysis *in vitro* using rat cerebellar membranes and was found to have a permissive effect on exogenous 2-AG administration in mice.[34] Though NAM is relatively selective for MAGL compared to FAAH and other serine hydrolases, NAM has limited use since the maleimide group is a thiol-reactive electrophile likely to react with many cysteine-containing residues. Indeed, CB1-knockout mice treated with NAM plus 2-AG administration retained locomotor inhibition similar to wild type mice, suggesting that NAM may have additional mechanisms of action. OMDM169, a derivative of tetrahydrolipostatin, was capable of a modest increase of 2-AG, but not anandamide, levels in neuroblastoma cells and in paws of formalin-treated mice. OMDM169 shared similar inhibitory effects for MAGL and pancreatic lipase while having an approximately 10-fold greater selectivity over FAAH and DAGLα.[35]

The sarin analog isopropyl dodecylfluorophosphonate (IDFP) and, surprisingly, the insecticide chlorpyrifos were also used to study the *in vivo* effects of inhibiting MAGL.[36] IDFP fully inhibited MAGL *in vivo*, but this inhibitor was non-selective, inhibiting MAGL, FAAH, and several other serine hydrolases. The insecticide chlorpyrifos completely blocked MAGL and partially blocked FAAH *in vivo* in the brain through bioactivation of this compound to chlorpyrifos oxon. While this insecticide was more selective than IDFP, it also inhibited the lethal target acetylcholinesterase. Both IDFP and chlorpyrifos administration showed many cannabinoid-mediated behaviors including catalepsy, which was later found to be caused by the dual blockade of

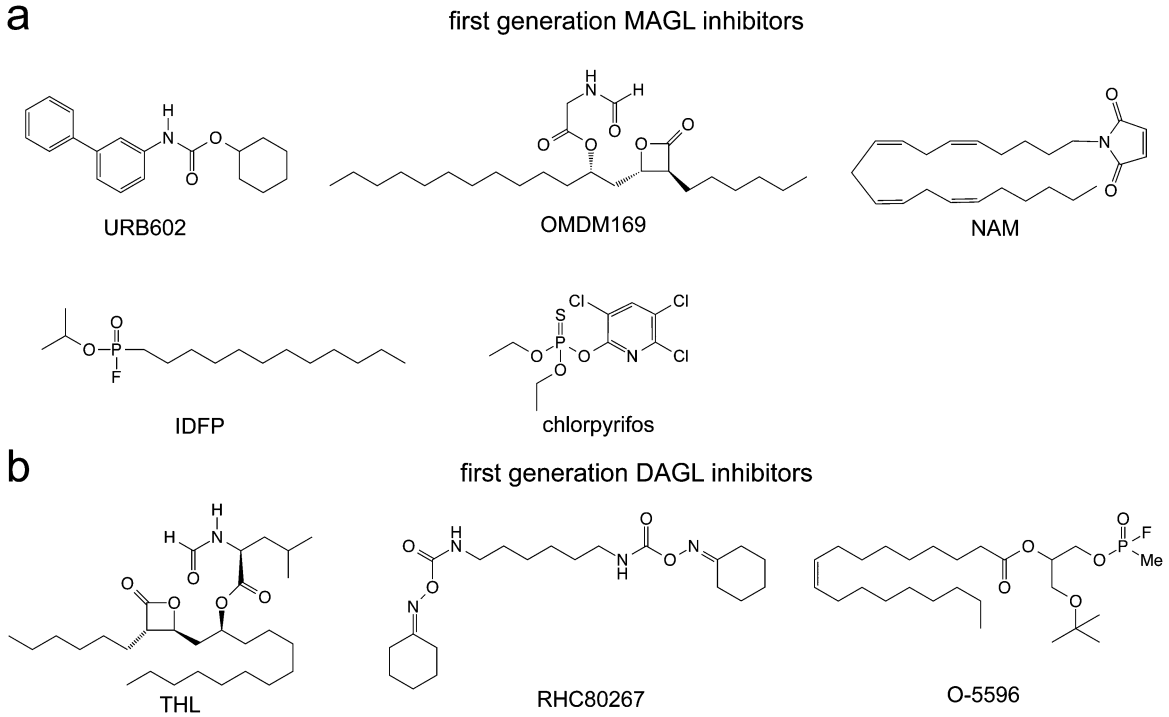

Fig. 2 First-generation MAGL and DAGL inhibitors. First-generation MAGL (a) and DAGL (b) inhibitors were non-selective, not potent, or not *in vivo* active.

MAGL and FAAH. IDFP and chlorpyrifos-treated mice showed > 10-fold elevation in brain 2-AG and anandamide levels, and interestingly also showed a stoichiometric reduction in arachidonic acid levels, indicating that 2-AG and arachidonic acid levels may be linked in the brain through MAGL.[36,37] Nonetheless, these inhibitors were limited in their ability to specifically dissect the roles of MAGL *in vivo* due to their non-selectivity.

3.2.2 First-generation DAGL inhibitors. The synthesis of dual DAGL inhibitors or selective DAGLα or DAGLβ inhibitors has been hampered by a lack of resolved crystal structures to provide structural knowledge about the target and a dearth of functional assays to assess endogenous DAGL activity. In early studies, *in vitro* hydrolysis of exogenous *sn*-1-[^{14}C]-oleoyl-2-arachidonoyl-glycerol was used as a readout of DAGL activity. The general lipase inhibitor tetrahydrolipostatin (THL, Orlistat) and the compound RHC-80267 inhibit DAGL-mediated synthesis of 2-AG, although at a higher concentration than needed to inhibit other lipases[15,38] (Fig. 2b). Both DAGL enzymes are also sensitive to treatment with the serine hydrolase inhibitors, mercury chloride, 4-chloromercuribenzoic acid and methyl arachidonoyl fluorophosphonate (MAFP).[39] Bisogno *et al.* also developed MAFP organophosphorus analogs, O-3640 and O-3841, which showed high selectivity for DAGLα over DAGLβ and other lipases but had poor potency, lack of stability, and poor cell penetration.[38] Further medicinal chemistry studies improved upon O-3841, yielding the similarly potent O-5596 but with better bioavailability and stability in physiological buffers.[40] Interestingly, O-5596-treated mice displayed a significant decrease in *ad libitum* consumption of sweetened cereal, but not regular chow, compared to vehicle-treated mice. This is in agreement with other studies using CB1 antagonists showing that endocannabinoid biosynthesis might be upregulated in response to palatable food exposure.[41,42] Indeed, animal models of overnutrition have been linked to elevated 2-AG levels, suggesting that DAGL inhibitors may be of use as anti-obesity therapeutics.[43]

3.3 Activity-based protein profiling (ABPP) for the development of DAGL and MAGL inhibitors

The identification and molecular characterization of more potent, selective, and *in vivo* efficacious inhibitors of MAGL and DAGL have been greatly accelerated by the use of the chemoproteomic technology, activity-based protein profiling (ABPP) (Fig. 3).[11,44,45] ABPP uses active-site directed chemical probes to directly assess the functional state of large numbers of enzymes in complex biological systems. Activity-based probes consist of a reactive chemical moiety that reacts with the active-site of specific enzyme class(es) and is coupled to an analytical handle, such as a fluorophore or biotin, enabling the detection of enzyme activities by fluorescence or mass-spectrometry based proteomics.[11,44–46] Because these probes bind to the active sites of enzymes, small-molecule inhibitor libraries can be competed directly against probe labeling of either pure enzymes or enzymes in complex native proteomes, enabling an assay strategy for inhibitor development. Furthermore, because the probe binds not only to the enzyme of interest, but also to the active-sites of other proteins in the enzyme class, ABPP facilitates the assessment of inhibitor selectivity on a proteome-wide scale. Additionally, with compounds that bind active sites irreversibly, target occupancy and selectivity of the inhibitors *in vivo* can be easily assessed *ex vivo* in any tissue of interest using ABPP platforms.[47]

Many of the enzymes involved in endocannabinoid metabolism, including MAGL, DAGL, and FAAH belong to the serine hydrolase superfamily of enzymes.[11,45] The fluorophosphonate (FP)-activity-based probes, FP-rhodamine and FP-biotin, were developed to assess the activities of serine hydrolases (Fig. 3a).[48] These and other activity based probes have been used successfully in developing selective FAAH, MAGL, and DAGL inhibitors (Fig. 3b). We will focus this review specifically on the development and effects of MAGL and DAGL inhibitors.

3.4 MAGL-selective inhibitors and their effects

Using ABPP platforms, Long *et al.* in 2009 put forth the first selective and *in vivo* active MAGL inhibitor JZL184, which contributed greatly in advancing our understanding of the physiological roles of MAGL (Fig. 4a).[49,50] JZL184 was developed through initial screening of a carbamate library of serine hydrolase inhibitors and subsequent optimization by traditional medicinal chemistry efforts. JZL184 is a piperidine carbamate that inhibits MAGL activity by irreversibly carbamylating the active-site catalytic serine nucleophile.[49,50] Competitive ABPP analysis using the FP-rhodamine probe revealed that JZL184 displayed 100-fold selectivity for MAGL over FAAH and was very selective against other mouse serine hydrolases expressed in the brain. Although highly selective in the brain, JZL184 had inhibitory effects on multiple carboxylesterase enzymes in peripheral tissues.[49,50] Inhibition of MAGL activity inhibited 2-AG hydrolysis by ~85% in mouse brain membranes and led to dramatic elevations in bulk brain 2-AG levels and increases in depolarization-induced interstitial 2-AG levels *in vivo*. These results confirmed that MAGL is the primary enzyme involved in degradation of 2-AG *in vivo*. A single dose of JZL184 at 16 mg kg^{-1} was capable of inhibiting MAGL for up to 24 h, with maximal 8-fold elevation of brain 2-AG levels for at least 8 hours.[49,50] Acute MAGL blockade with JZL184 has been shown to exhibit a wide range of beneficial effects including alleviation of pain, inflammation, emesis, anxiety, opiate-induced withdrawal symptoms, colitis, neurodegeneration, inflammation-induced lung and liver injury, and cancer pathogenicity.[21,51–55] These effects are discussed further below. Interestingly, MAGL inhibitors do not cause full-blown cannabinoid behaviors such as hypothermia and catalepsy, although they lower motility in open-field tests in mice despite apparently normal cage behavior.[49,50] Chronic and complete pharmacological blockade of MAGL, as observed in MAGL −/− mice, leads to functional antagonism of the cannabinoid system, leading to a loss of cannabinoid-mediated effects, physical dependency, and desensitization of CB1 receptors in the brain. Thus, the MAGL inhibitor has been especially useful compared to full genetic knockout mouse models, since the cannabinoid effects are ablated upon chronic and complete inactivation of MAGL in MAGL −/− mice.[27]

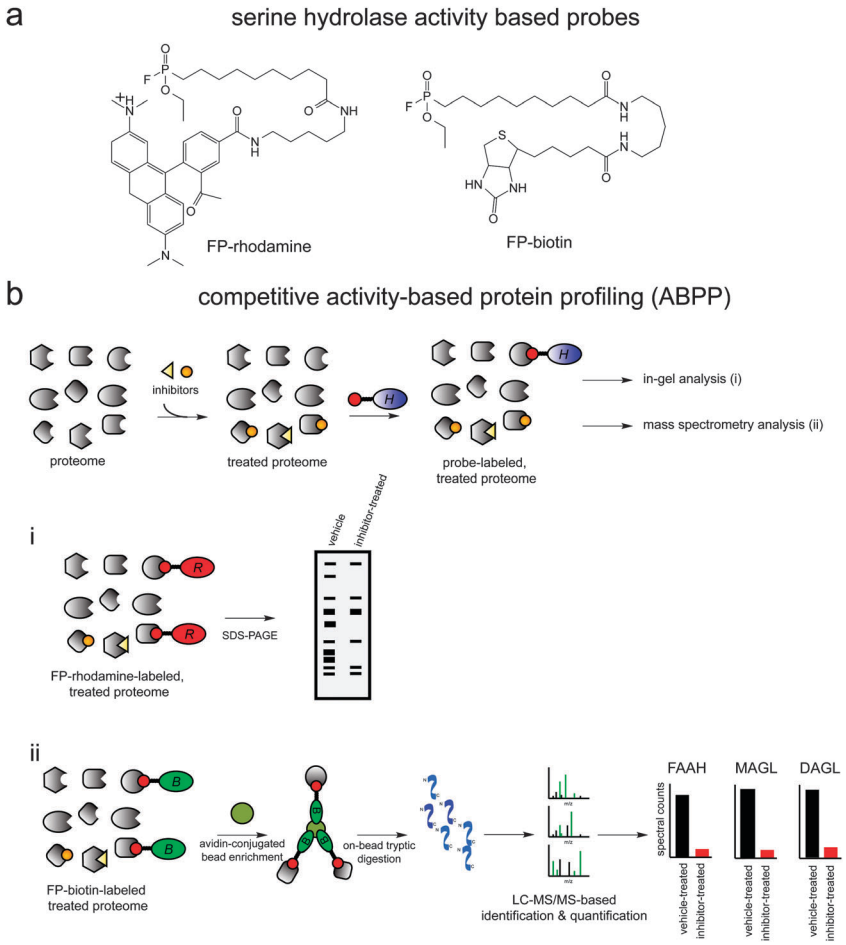

Fig. 3 Activity-based protein profiling (ABPP) for developing MAGL and DAGL inhibitors. (a) Activity-based probes for serine hydrolases, fluorophosphonate (FP)-rhodamine and FP-biotin. (b) Competitive ABPP has been used to develop highly selective MAGL and DAGL inhibitors. ABPP use active-site directed probes conjugated to an analytical handle (H) to assess the activities of large numbers of enzymes either by in-gel fluorescence using a rhodamine (R)-bound probe (i) or by mass-spectrometry-based proteomics using a biotin (B)-bound probe (ii). Because the probes bind to the active-sites, small-molecule inhibitor libraries can be competed against probe binding, facilitating an inhibitor-discovery platform. Selectivity can also be assessed across the entire enzyme class since probe-bound enzyme activities can be read-out in parallel.

Subsequent studies have shown that partial and chronic blockade of MAGL avoids this functional antagonism of CB1 and thus maintains the cannabinoid-mediated effects.[56]

Recent studies have yielded next-generation MAGL inhibitors with improved selectivity and cross-species activity compared to JZL184. These include the *O*-hexafluoroisopropyl carbamates and the *N*-hydroxysuccinimidyl (NHS) carbamates. The *O*-hexafluoroisopropyl leaving group on the newer MAGL inhibitors displayed greater selectivity towards MAGL over FAAH and, importantly, carboxylesterase enzymes both *in vitro* and *in vivo*. KML29, an *O*-hexafluoroisopropyl analog of JZL184, was completely selective for MAGL over FAAH even in chronically dosed mice using ABPP. This hexafluoroisopropyl leaving group of KML29 was found to be bioisosteric with the 2-AG substrate, indicating that serine hydrolase inhibitor selectivity may be better achieved my developing inhibitors bearing reactive groups resembling the structures of endogenous substrates.[57] JZL184 had limited efficacy toward rat MAGL both *in vitro* and *in vivo*. In contrast, KML29 treatment showed near complete MAGL blockade and increased brain 2-AG levels in rats.[57] A subsequent report also showed that a close analog of KML29, JW651, also selectively inhibited MAGL *in vitro* and *in vivo*.[58] Niphakis *et al.* reported MNJ110 as a highly potent, selective, and *in vivo* active NHS carbamate inhibitor of MAGL (Fig. 4a).[58]

Both Niphakis *et al.* and Chang *et al.* also recently used click-chemistry-ABPP using alkyne-bearing "clickable" analogs of highly selective MAGL inhibitors to confirm the selectivity of these compounds across the entire proteome by comprehensively mining all covalent probe–protein interactions (Fig. 4a).[58,59] The alkyne-bearing inhibitor protein targets are detected by conjugation with a rhodamine–azide reporter tag using copper-catalyzed azide–alkyne cycloaddition chemistry. The click-chemistry carbamate probes, such as JW651yne and MJN110yne, showed selective labeling of MAGL at low concentrations with FAAH, ABHD6, as well as other enzymes not detected by ABPP, as off-targets at higher concentrations.[59]

Chang *et al.* also developed a fluorescent imaging probe for MAGL and ABHD6, based on the *O*-hexafluoroisopropyl carbamates

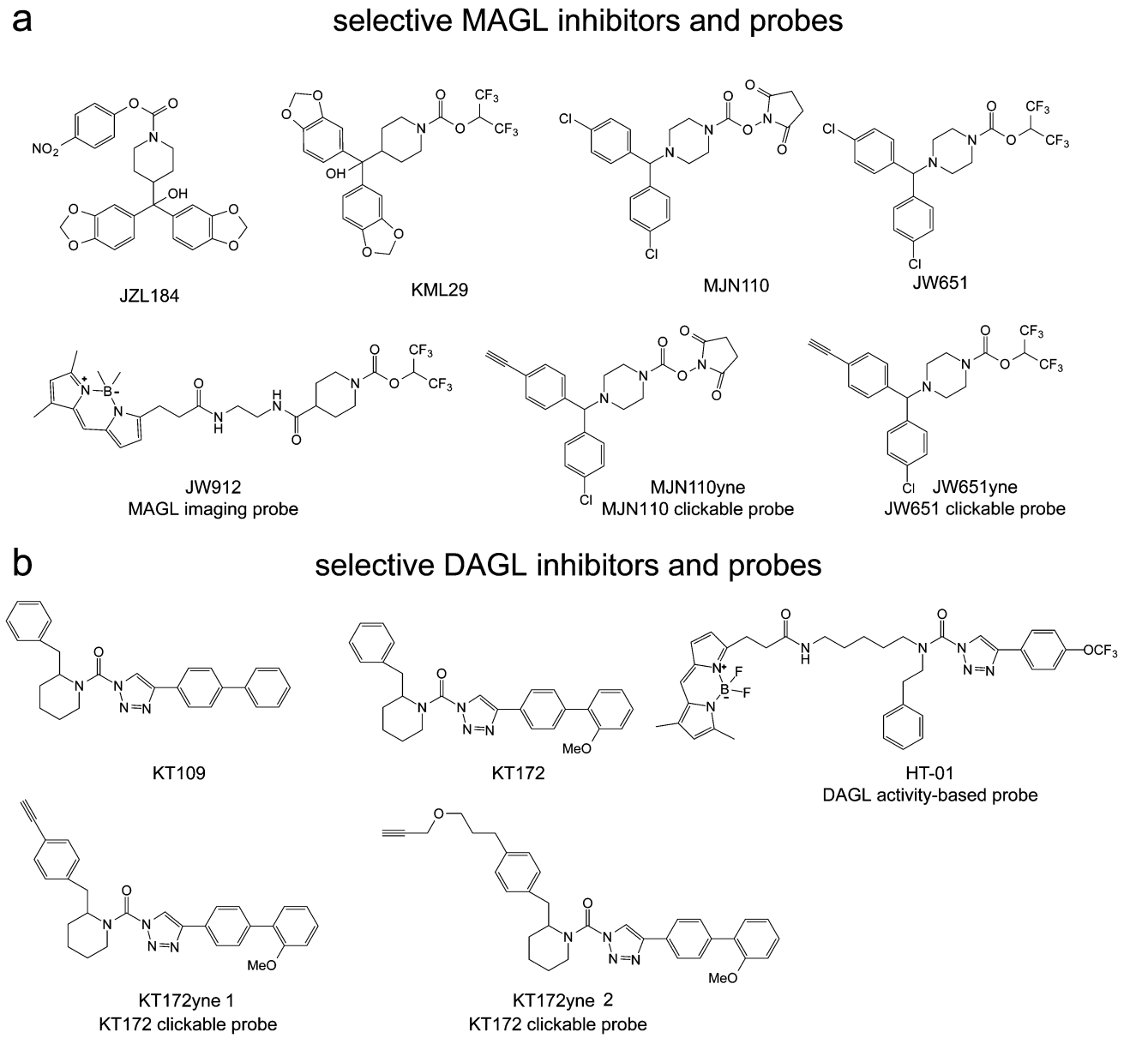

Fig. 4 Selective MAGL and DAGL inhibitors and probes. Selective MAGL (a: JZL184, KML29, MJN110, JW651) and DAGL (b: KT109, KT172) inhibitors, MAGL imaging probe (JW912), MAGL and DAGL clickable probes (MJN110yne, JW651yne, KT172yne 1 and 2), and the DAGL activity-based probe (HT-01).

that showed high specificity to these two enzymes, resulting in the BODIPY-containing *O*-hexafluoroisopropyl carbamate JW912 (Fig. 4a). This probe selectively labeled MAGL and ABHD6 as determined by in-gel fluorescence and the authors subsequently used this probe to visualize MAGL and ABHD6 localization in various cancer cells. To distinguish cellular localization of MAGL versus ABHD6, the authors used MAGL and ABHD6-selective inhibitors. They showed that MAGL in certain cancer cells display fluorescent signal on intracellular membranes and show punctate staining patterns indicating localization on endosomes and other organelles.[59]

Collectively, these selective MAGL inhibitors have been invaluable in elucidating the biochemical and physiological roles of MAGL *in vivo* as well as in establishing the therapeutic potential of MAGL inhibitors in various pathological states. The insights attained from these inhibitors are described in more detail below.

3.4.1 MAGL also controls arachidonic acid pools that generate pro-inflammatory eicosanoids in select tissues. In addition to the role of MAGL in terminating 2-AG signaling, studies using both non-selective and highly selective MAGL inhibitors and MAGL knockout mice have found that MAGL is the primary source of arachidonic acid for the generation of pro-inflammatory eicosanoids in certain tissues, including the brain, the liver, and the lungs.[51] Both pharmacological blockade with JZL184 and genetic ablation of MAGL lower basal and lipopolysaccharide-induced arachidonic acid and eicosanoid levels in these tissues. These results are surprising, since cytosolic phospholipase A2 (cPLA2)

has been historically considered to be the primary source of arachidonic acid for eicosanoid synthesis.[51] Using MAGL inhibitors and MAGL −/− and cPLA2 −/− mice, Nomura *et al.* found that MAGL contributes ~80% of LPS-stimulated eicosanoids in the mouse brain while cPLA2 contributes ~20%. However, in the spleen and in the gastrointestinal tract, the authors showed that cPLA2 is the dominant enzyme that controls arachidonic acid release for prostaglandin production. Thus, MAGL, cPLA2, and potentially other enzymes differentially control arachidonic acid release in a tissue-specific manner.[51]

3.4.2 The effect of MAGL inhibitors in pain, inflammation, and mood. MAGL blockade with JZL184 has been shown in many studies to elicit CB1-dependent antinociceptive effects in various mouse models of pain, including noxious chemical, inflammatory, thermal, and neuropathic pain.[49,60,61] MAGL blockade reduces mechanical and acetone-induced cold allodynia in mice with sciatic nerves that had previously undergone chronic constriction injury.[60] MAGL blockade is also protective in mouse models of inflammatory bowel disease, in which MAGL blockade by JZL184 reduces colon cytopathology, inflammatory cytokine levels, and restores intestinal barrier function in a trinitrobenzene sulfonic acid-induced colitis model, thereby reducing endotoxemia and systemic inflammation in a CB1 or CB2-dependent manner.[62]

Multiple studies have shown that MAGL blockade by JZL184 also exerts effects upon mood and reward behavior. In a marble burying model of repetitive and compulsive behavior inherent to anxiety disorders, MAGL blockade reduced marble burying.[63] MAGL blockade also exerts anxiolytic effects in an elevated plus-maze paradigm for anxiety.[64] Chronic MAGL blockade with JZL184 also prevented chronic stress-induced anxiety-like behavior and long-term depression of GABAergic transmission, indicating that MAGL inhibition prevents behavioral and synaptic adaptations to chronic stress that may lead to the worsening of affective disorders.[65] MAGL inhibitors also improve withdrawal symptoms from naloxone-precipitated morphine withdrawal in a CB1-dependent manner.[66]

3.4.3 The effect of MAGL inhibitors in neuroinflammation and neurodegenerative diseases. Both pharmacological and genetic ablation of MAGL show anti-inflammatory effects in the brain and neuroprotective effects in mouse models of Parkinson's and Alzheimer's disease.[51,53,54] MAGL inhibition lowers LPS-stimulated pro-inflammatory cytokine levels in the brain by lowering neuroinflammatory eicosanoids, in a CB1 and CB2-independent manner.[51] MAGL blockade with JZL184 or MAGL deficiency also protects against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced dopaminergic neurodegeneration and dopamine loss by lowering pro-inflammatory eicosanoids and suppressing neuroinflammation.[51]

Two studies recently showed that MAGL inhibition with JZL184 or MAGL deficiency both lower amyloid-β plaque levels in Alzheimer's disease mouse models in-part by lowering eicosanoids and suppressing microglial and astrocyte activation.[53,54] Piro *et al.* showed that MAGL −/− mice crossed with the presenilin/amyloid precursor peptide (PS/APP) transgenic Alzheimer's disease mouse model possessed significantly lower amyloid-β peptide and plaque levels concomitant with reduced neuroinflammation. JZL184 administration recapitulated the lowering of inflammatory cytokines in PS/APP-transgenic mice and CB1 or CB2 receptor antagonists did not attenuate this reduction. Chen *et al.* also showed that JZL184 robustly suppressed the production and accumulation of amyloid-β by downregulating β-site amyloid precursor protein cleaving enzyme 1, concomitant with a suppression of neuroinflammation, in the 5X FAD APP transgenic mice. They also confirmed that this phenomenon was CB1 and CB2-independent. Quite provocatively, Chen *et al.* also showed that JZL184 also reduced neurodegeneration, maintained the integrity of hippocampal synaptic structure and function, and improved long-term synaptic plasticity, spatial learning, and memory in this Alzheimer's disease mouse model.

Thus, several studies have shown the potential therapeutic utility of MAGL inhibitors in attenuating neuroinflammation and protecting against neurodegeneration in both Parkinson's and Alzheimer's disease mouse models and indicate that MAGL inhibitors may even improve memory and learning function, likely by lowering the arachidonic acid and pro-inflammatory eicosanoid levels in the brain.

3.4.4 The effect of MAGL inhibitors on inflammatory tissue injury. Recent studies have also shown that MAGL inhibitors may have therapeutic windows not only in neuroinflammatory or neurodegenerative diseases, but also in peripheral inflammatory tissue injury as well. Cao *et al.* showed that the endocannabinoid and eicosanoid levels in the liver are elevated upon ischemia-reperfusion injury in mice and that pharmacological or genetic MAGL inactivation significantly protects against hepatocellular cell death as evidenced by lower hepatic necrosis, reduction in liver-damage blood serum markers ALT and AST, and lower levels of liver cell death markers. MAGL inactivation also lowered hepatic inflammation caused by ischemia-reperfusion injury by lowering neutrophil infiltration, inflammatory cytokines, and reactive oxygen stress. Quite intriguingly, in contrast to the previously described models where the phenotypes observed were either due to enhanced CB1/CB2 signaling or lower eicosanoid levels, this hepatoprotective phenotype appeared to be due to a combination of enhanced CB2 signaling *and* lower eicosanoid levels. The authors also provocatively demonstrated that JZL184 could even protect against liver injury when provided 3 h after reperfusion. Cao *et al.* also showed that JZL184 was protective in the carbon tetrachloride and galactosamine/LPS models of liver injury in mice.[52]

Costola-de-Souza *et al.* recently showed that JZL184 protected against lung injury in a LPS-induced acute lung injury model. The authors showed that an acute treatment with JZL184 reduced leukocyte migration into the lungs, vascular permeability, and inflammatory cytokine and chemokine levels in bronchoalveolar lavage fluid. These protective effects appeared to be mediated through CB1 and CB2 receptors, as the effects were attenuated with CB1 and CB2 selective antagonists.[55]

3.4.5 The effect of MAGL inhibitors on cancer. Using ABPP platforms, Nomura *et al.* showed that MAGL is highly upregulated across multiple types of aggressive human cancer cells and

primary high-grade tumors.[67,68] Both genetic and pharmacological ablation of MAGL in aggressive cancer cells impaired cellular migration, invasiveness, serum-free cell survival, and *in vivo* tumor growth. Metabolomic analysis showed that MAGL in aggressive cancer cells controls the lipolytic release of free fatty acids which are in turn remodeled into various lysophosholipids and eicosanoids. MAGL blockade lowered cellular fatty acid levels and downstream tumor-promoting lipid signaling molecules such as eicosanoids and lysophosphatidic acid, leading to impairments in cancer pathogenicity.[67,68] In ovarian, breast, and melanoma cancer cells, MAGL inactivation produced anti-cancer effects by reducing the fatty acid network of oncogenic signaling lipids, and not through CB1 or CB2-dependent mechanisms. In contrast, in aggressive prostate cancer cells, the anti-tumorigenic phenotypes associated with MAGL blockade were due to a combination of heightened CB1 signaling and reduced fatty acid and fatty acid-derived lipid signaling.[67,68] Ye *et al.* also showed that MAGL blockade impairs colorectal cancer cell pathogenicity and tumor growth through downregulation of cyclin D1 and Bcl-2.[69]

MAGL blockade has also been shown to alleviate pain associated with cancer through heightened CB2 signaling in a mouse model of mechanical hyperalgesia evoked by the growth of a fibrosarcoma tumor in the calcaneus bone.[70] MAGL blockade also shows anti-emetic effects in a lithium chloride model of vomiting.[71]

3.5 DAGL-selective inhibitors and their effects

Recently, the first specific and *in vivo* active DAGLβ inhibitors were reported, based on the triazole urea scaffold (Fig. 4b).[72,73] Using competitive ABPP platforms, Hsu *et al.* screened recombinantly expressed DAGL enzymes against a library of 1,2,3-triazole urea inhibitors, a chemotype that was previously shown to possess well-suited features for serine hydrolase inhibitor development. Two compounds from this screen, KT109 and KT172, potently inhibited DAGLβ with a ~60-fold selectivity over DAGLα. These inhibitors showed high selectivity for DAGLβ over other serine hydrolases, but both inhibitors showed ABHD6 as an off-target. KT109 and KT172 inhibit DAGLβ with an IC_{50} of 82 and 71 nM, respectively. At higher concentrations, KT109 and KT172 showed some inhibitory activity against PLA2G7 (IC_{50} 1000 nM) and MAGL (IC_{50} 5000 nM), respectively. To exclude the effects of ABHD6 off-target from their studies, Hsu *et al.* also developed a control inhibitor KT195 that was a close structural analog of KT109 and KT172 that did not inhibit DAGLβ but inhibited ABHD6.[72,73]

While the serine hydrolase activity-based FP-rhodamine probes were able to easily assess recombinantly overexpressed DAGL activity, the low endogenous expression level of DAGLβ in cells and tissues prohibited its detection by broad-based probes such as FP-rhodamine. To easily confirm target-engagement of KT109 and KT172 *in vitro*, *in situ*, and *in vivo*, Hsu *et al.* also developed a tailored activity-based probe for DAGLβ based on the 1,2,3-triazole urea scaffold, HT-01, a BODIPY-conjugated 1,2,3-triazole urea probe that selectively labeled endogenous DAGLβ in complex proteomes (Fig. 4b). Using this HT-01 probe, Hsu *et al.* showed that KT109 and KT172 inhibited DAGLβ *in situ* in Neuro2A cells and *in vivo* in mouse macrophages. The authors also used ABPP using the FP-biotin probe coupled to proteomic-based methods to confirm target occupancy and selectivity of KT109 and KT172 *in situ* and *in vivo*. Hsu *et al.* in a subsequent study also developed "clickable" analogs of KT172, confirming the selectivity of this inhibitor for DAGLβ and ABHD6 using click-chemistry-ABPP (Fig. 4b).[72,73]

3.5.1 Effects of DAGL inhibitors on endocannabinoids, eicosanoids, and inflammation. KT109 and KT172 were used to show that DAGLβ blockade lowers the levels of 2-AG, arachidonic acid, and prostaglandins in Neuro2A cells, mouse peritoneal macrophages, mouse liver, and human prostate cancer cells, indicating that the DAGL/MAGL and cPLA2 pathways both play complementary roles in arachidonic acid release for eicosanoid biosynthesis. While DAGLβ blockade alone lowers LPS-stimulated TNF-α release, which was also recapitulated in DAGLβ-deficient mice, DAGLβ and cPLA2 dual inactivation leads to an increase in TNF-α release.[72]

Until now, understanding the role of DAGLs in mammalian physiology and pathophysiology has been hindered by lack of inhibitors that are not just specific to DAGLs over other serine hydrolases, but are specific to only one isoform. These newest DAGL inhibitors will be immensely useful in future studies for understanding the nodal role of DAGL in regulating diacylglycerol, endocannabinoid, and eicosanoid signaling networks.

4. Potential liabilities of DAGL and MAGL inhibitors

With the many beneficial effects associated with DAGL and MAGL blockade, a key question is whether there may be any adverse effects associated with DAGL and MAGL inhibitors. One potential liability that may be associated with DAGL inhibitors is a potential impairment in adult neurogenesis as has been shown in DAGL knockout mice.[17] Another potential adverse effect that may arise from DAGL blockade may be those phenotypes associated with functional antagonism of CB1. Previous studies have shown that CB1 antagonists show beneficial effects towards weight loss and improved serum lipid profiles, insulin sensitivity, and cardiometabolic parameters.[74] However, CB1 antagonists were discontinued and clinical trials were terminated due to increased anxiety and depression.[75] One can conceivably avoid these potential adverse effects by developing either reversible DAGL inhibitors or small-molecules that do not cross the blood–brain barrier.

Liabilities that may be associated with MAGL inhibitors also include potential psychiatric effects that may arise from functional antagonism of CB1 in the brain. Previous studies have demonstrated that complete and chronic blockade of MAGL leads to a functional antagonism of CB1 in the brain, leading to reduced sensitivity to exogenous CB1 agonists and physical dependence as well as a loss of CB1-mediated phenotypes associated with acute MAGL blockade.[27] This occurs due to

prolonged heightening of 2-AG levels and CB1 stimulation, leading to desensitized brain CB1.

These potential adverse effects may be avoided by either lowering the dose of currently available irreversible inhibitors to ensure that MAGL is not completely inactivated or by developing potent and selective reversible MAGL inhibitors. Kinsey *et al.* have already shown that repeated low-dose administration of JZL184 retains CB1-mediated antinociceptive and gastroprotective effects in mice. Cisneros *et al.* recently described the structure–activity relationship of a new series of reversible dual MAGL and FAAH inhibitors (±)-oxiran-2-ylmethyl 6-(1,1′-biphenyl-4-yl)hexanoate and (2*R*)-(−)-oxiran-2-ylmethyl(4-benzylphenyl)acetate. While the selectivity of these inhibitors across other proteins is not known, developing reversible and selective MAGL inhibitors will be of future importance in understanding the therapeutic potential of MAGL inhibitors in relation to the currently available selective irreversible inhibitors.

Furthermore, it is important that any future MAGL inhibitor therapy that crosses the blood–brain barrier be selective for MAGL over FAAH, since studies have shown that dual MAGL and FAAH blockade results in effects reminiscent of THC, such as catalepsy, not observed with either MAGL or FAAH inhibition alone.[76]

5. Future therapeutic potential of MAGL and DAGL inhibitors

There are still many unanswered questions in the endocannabinoid field that will hopefully be addressed with the development and utilization of even more advanced MAGL and DAGL inhibitors. While Hsu *et al.* developed the first selective and *in vivo* active DAGLβ inhibitors (that also show DAGLα inhibition at higher concentrations), KT172 does not appear to cross the blood–brain barrier.[72] Thus, it will be of future interest to develop highly selective *in vivo* active and brain penetrant DAGLα inhibitors and to test their effects upon memory, synaptic plasticity, neuroinflammation, and in neurodegenerative disease models. With previous studies showing the importance of the endocannabinoid system in satiety, lipid metabolism, obesity, diabetes, and cardiovascular disease, it will also be of future interest to understand the effects of the newer and selective MAGL and DAGL inhibitors on obesity and diabetes paradigms.

With the diverse roles of diacylglycerol, 2-AG, and eicosanoid signaling pathways, DAGL and MAGL inhibitors are likely to be critical to future investigations into dissecting the individual roles of these lipid signaling pathways as well as the complementary roles of cPLA2 and other phospholipases in eicosanoid metabolism, signaling, and associated (patho)physiological effects.

We have reviewed here the use of modern chemical proteomic technologies such as ABPP in developing highly selective inhibitors for 2-AG degradation and synthesis. Studies using highly selective and *in vivo* active MAGL inhibitors have shown that these inhibitors may have potential therapeutic utility towards attenuating pain, inflammation, drug-withdrawal symptoms, anxiety, neurodegenerative diseases, ischemia-reperfusion tissue injuries, inflammation-induced injuries in the liver and lung, and cancer and cancer-associated symptoms. DAGLβ inhibitors have been shown to elicit anti-TNF-α effects, which may have therapeutic utility in inflammatory disease such as rheumatoid arthritis where anti-TNF-α antibodies have shown favorable effects (Fig. 5). The next steps in the clinical development of MAGL and DAGL inhibitors will be to test their toxicological properties, optimize pharmacokinetic parameters, and further show their efficacy in pre-clinical models towards advancing these inhibitors to the clinic to treat various human diseases that show dysregulated diacylglycerol, endocannabinoid, or eicosanoid signaling pathways.

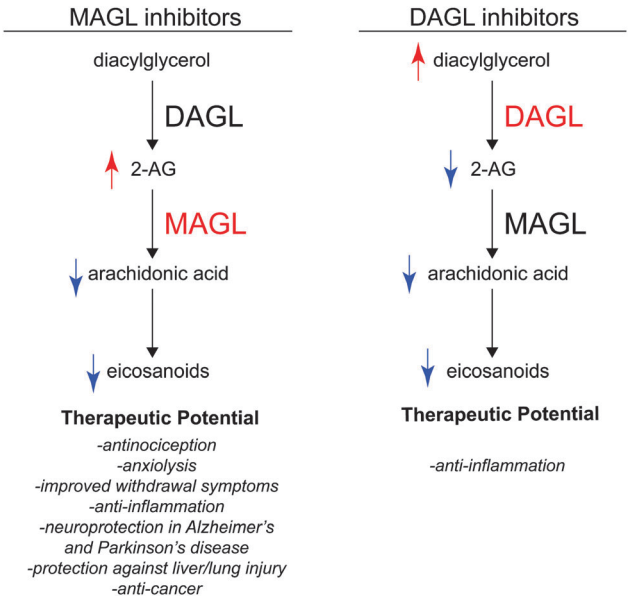

Fig. 5 Metabolic and biological effects of MAGL and DAGL inhibitors.

Acknowledgements

We thank the members of the Nomura Research Group for critical reading of the manuscript. This work was supported by grants from the National Institutes of Health (R00DA030908), the Searle Scholar Foundation, and Michael J. Fox Target Validation Award.

References

1 I. B. Adams and B. R. Martin, *Br. J. Addict.*, 1996, **91**, 1585–1614.
2 V. Di Marzo, T. Bisogno and L. De Petrocellis, *Chem. Biol.*, 2007, **14**, 741–756.
3 V. Di Marzo and S. Petrosino, *Curr. Opin. Lipidol.*, 2007, **18**, 129–140.
4 K. Ahn, M. K. McKinney and B. F. Cravatt, *Chem. Rev.*, 2008, **108**, 1687–1707.
5 B. E. Alger and J. Kim, *Trends Neurosci.*, 2011, **34**, 304–315.

6 J. C. Ashton and M. Glass, *Curr. Neuropharmacol.*, 2007, **5**, 73–80.
7 E. V. Berdyshev, *Chem. Phys. Lipids*, 2000, **108**, 169–190.
8 J. M. Derocq, M. Segui, J. Marchand, G. Lefur and P. Casellas, *FEBS Lett.*, 1995, **369**, 177–182.
9 S. Galiegue, S. Mary, J. Marchand, D. Dussossoy, D. Carriere, P. Carayon, M. Bouaboula, D. Shire, G. Le Fur and P. Casellas, *Eur. J. Biochem.*, 1995, **232**, 54–61.
10 A. M. Miller and N. Stella, *Br. J. Pharmacol.*, 2008, **153**, 299–308.
11 J. L. Blankman and B. F. Cravatt, *Pharmacol. Rev.*, 2013, **65**, 849–871.
12 A. C. Howlett, L. C. Blume and G. D. Dalton, *Curr. Med. Chem.*, 2010, **17**, 1382–1393.
13 B. Pan, W. Wang, P. Zhong, J. L. Blankman, B. F. Cravatt and Q. S. Liu, *J. Neurosci.*, 2011, **31**, 13420–13430.
14 T. Sugiura, S. Kondo, A. Sukagawa, S. Nakane, A. Shinoda, K. Itoh, A. Yamashita and K. Waku, *Biochem. Biophys. Res. Commun.*, 1995, **215**, 89–97.
15 N. Stella, P. Schweitzer and D. Piomelli, *Nature*, 1997, **388**, 773–778.
16 B. Pan, W. Wang, J. Z. Long, D. Sun, C. J. Hillard, B. F. Cravatt and Q. S. Liu, *J. Pharmacol. Exp. Ther.*, 2009, **331**, 591–597.
17 Y. Gao, D. V. Vasilyev, M. B. Goncalves, F. V. Howell, C. Hobbs, M. Reisenberg, R. Shen, M. Y. Zhang, B. W. Strassle, P. Lu, L. Mark, M. J. Piesla, K. Deng, E. V. Kouranova, R. H. Ring, G. T. Whiteside, B. Bates, F. S. Walsh, G. Williams, M. N. Pangalos, T. A. Samad and P. Doherty, *J. Neurosci.*, 2010, **30**, 2017–2024.
18 H. Yoshino, T. Miyamae, G. Hansen, B. Zambrowicz, M. Flynn, D. Pedicord, Y. Blat, R. S. Westphal, R. Zaczek, D. A. Lewis and G. Gonzalez-Burgos, *J. Physiol.*, 2011, **589**, 4857–4884.
19 A. Tanimura, M. Yamazaki, Y. Hashimotodani, M. Uchigashima, S. Kawata, M. Abe, Y. Kita, K. Hashimoto, T. Shimizu, M. Watanabe, K. Sakimura and M. Kano, *Neuron*, 2010, **65**, 320–327.
20 R. Min, V. Di Marzo and H. D. Mansvelder, *Neuroscientist*, 2010, **16**, 608–613.
21 M. M. Mulvihill and D. K. Nomura, *Life Sci.*, 2013, **92**, 492–497.
22 T. P. Dinh, D. Carpenter, F. M. Leslie, T. F. Freund, I. Katona, S. L. Sensi, S. Kathuria and D. Piomelli, *Proc. Natl. Acad. Sci. U. S. A.*, 2002, **99**, 10819–10824.
23 T. P. Dinh, S. Kathuria and D. Piomelli, *Mol. Pharmacol.*, 2004, **66**, 1260–1264.
24 M. Karlsson, J. A. Contreras, U. Hellman, H. Tornqvist and C. Holm, *J. Biol. Chem.*, 1997, **272**, 27218–27223.
25 H. Tornqvist and P. Belfrage, *J. Biol. Chem.*, 1976, **251**, 813–819.
26 J. L. Blankman, G. M. Simon and B. F. Cravatt, *Chem. Biol.*, 2007, **14**, 1347–1356.
27 J. E. Schlosburg, J. L. Blankman, J. Z. Long, D. K. Nomura, B. Pan, S. G. Kinsey, P. T. Nguyen, D. Ramesh, L. Booker, J. J. Burston, E. A. Thomas, D. E. Selley, L. J. Sim-Selley, Q. S. Liu, A. H. Lichtman and B. F. Cravatt, *Nat. Neurosci.*, 2010, **13**, 1113–1119.
28 G. Thomas, J. L. Betters, C. C. Lord, A. L. Brown, S. Marshall, D. Ferguson, J. Sawyer, M. A. Davis, J. T. Melchior, L. C. Blume, A. C. Howlett, P. T. Ivanova, S. B. Milne, D. S. Myers, I. Mrak, V. Leber, C. Heier, U. Taschler, J. L. Blankman, B. F. Cravatt, R. G. Lee, R. M. Crooke, M. J. Graham, R. Zimmermann, H. A. Brown and J. M. Brown, *Cell Rep.*, 2013, **5**, 508–520.
29 J. L. Blankman, J. Z. Long, S. A. Trauger, G. Siuzdak and B. F. Cravatt, *Proc. Natl. Acad. Sci. U. S. A.*, 2013, **110**, 1500–1505.
30 S. M. Saario, J. R. Savinainen, J. T. Laitinen, T. Jarvinen and R. Niemi, *Biochem. Pharmacol.*, 2004, **67**, 1381–1387.
31 A. G. Hohmann, R. L. Suplita, N. M. Bolton, M. H. Neely, D. Fegley, R. Mangieri, J. F. Krey, J. M. Walker, P. V. Holmes, J. D. Crystal, A. Duranti, A. Tontini, M. Mor, G. Tarzia and D. Piomelli, *Nature*, 2005, **435**, 1108–1112.
32 G. G. Muccioli, C. Xu, E. Odah, E. Cudaback, J. A. Cisneros, D. M. Lambert, M. L. Lopez Rodriguez, S. Bajjalieh and N. Stella, *J. Neurosci.*, 2007, **27**, 2883–2889.
33 S. Vandevoorde, K. O. Jonsson, G. Labar, E. Persson, D. M. Lambert and C. J. Fowler, *Br. J. Pharmacol.*, 2007, **150**, 186–191.
34 J. J. Burston, L. J. Sim-Selley, J. P. Harloe, A. Mahadevan, R. K. Razdan, D. E. Selley and J. L. Wiley, *J. Pharmacol. Exp. Ther.*, 2008, **327**, 546–553.
35 T. Bisogno, G. Ortar, S. Petrosino, E. Morera, E. Palazzo, M. Nalli, S. Maione, V. Di Marzo and E. R. Grp, *Biochim. Biophys. Acta, Mol. Cell Biol. Lipids*, 2009, **1791**, 53–60.
36 D. K. Nomura, J. L. Blankman, G. M. Simon, K. Fujioka, R. S. Issa, A. M. Ward, B. F. Cravatt and J. E. Casida, *Nat. Chem. Biol.*, 2008, **4**, 373–378.
37 D. K. Nomura, C. S. Hudak, A. M. Ward, J. J. Burston, R. S. Issa, K. J. Fisher, M. E. Abood, J. L. Wiley, A. H. Lichtman and J. E. Casida, *Bioorg. Med. Chem. Lett.*, 2008, **18**, 5875–5878.
38 T. Bisogno, M. G. Cascio, B. Saha, A. Mahadevan, P. Urbani, A. Minassi, G. Appendino, C. Saturnino, B. Martin, R. Razdan and V. Di Marzo, *Biochim. Biophys. Acta*, 2006, **1761**, 205–212.
39 T. Bisogno, F. Howell, G. Williams, A. Minassi, M. G. Cascio, A. Ligresti, I. Matias, A. Schiano-Moriello, P. Paul, E. J. Williams, U. Gangadharan, C. Hobbs, V. Di Marzo and P. Doherty, *J. Cell Biol.*, 2003, **163**, 463–468.
40 T. Bisogno, J. J. Burston, R. Rai, M. Allara, B. Saha, A. Mahadevan, R. K. Razdan, J. L. Wiley and V. Di Marzo, *ChemMedChem*, 2009, **4**, 946–950.
41 V. Di Marzo and I. Matias, *Nat. Neurosci.*, 2005, **8**, 585–589.
42 T. C. Kirkham, C. M. Williams, F. Fezza and V. Di Marzo, *Br. J. Pharmacol.*, 2002, **136**, 550–557.
43 V. Di Marzo, S. K. Goparaju, L. Wang, J. Liu, S. Batkai, Z. Jarai, F. Fezza, G. I. Miura, R. D. Palmiter, T. Sugiura and G. Kunos, *Nature*, 2001, **410**, 822–825.
44 D. A. Bachovchin and B. F. Cravatt, *Nat. Rev. Drug Discovery*, 2012, **11**, 52–68.
45 G. M. Simon and B. F. Cravatt, *J. Biol. Chem.*, 2010, **285**, 11051–11055.
46 D. K. Nomura, M. M. Dix and B. F. Cravatt, *Nat. Rev. Cancer*, 2010, **10**, 630–638.

47 D. Medina-Cleghorn and D. K. Nomura, *Pfluegers Arch.*, 2013, **465**, 427–440.
48 Y. Liu, M. P. Patricelli and B. F. Cravatt, *Proc. Natl. Acad. Sci. U. S. A.*, 1999, **96**, 14694–14699.
49 J. Z. Long, W. Li, L. Booker, J. J. Burston, S. G. Kinsey, J. E. Schlosburg, F. J. Pavon, A. M. Serrano, D. E. Selley, L. H. Parsons, A. H. Lichtman and B. F. Cravatt, *Nat. Chem. Biol.*, 2009, **5**, 37–44.
50 J. Z. Long, D. K. Nomura and B. F. Cravatt, *Chem. Biol.*, 2009, **16**, 744–753.
51 D. K. Nomura, B. E. Morrison, J. L. Blankman, J. Z. Long, S. G. Kinsey, M. C. Marcondes, A. M. Ward, Y. K. Hahn, A. H. Lichtman, B. Conti and B. F. Cravatt, *Science*, 2011, **334**, 809–813.
52 Z. Cao, M. M. Mulvihill, P. Mukhopadhyay, H. Xu, K. Erdelyi, E. Hao, E. Holovac, G. Hasko, B. F. Cravatt, D. K. Nomura and P. Pacher, *Gastroenterology*, 2013, **144**, 808–817, e815.
53 J. R. Piro, D. I. Benjamin, J. M. Duerr, Y. Pi, C. Gonzales, K. M. Wood, J. W. Schwartz, D. K. Nomura and T. A. Samad, *Cell Rep.*, 2012, **1**, 617–623.
54 R. Q. Chen, J. Zhang, Y. Wu, D. Q. Wang, G. P. Feng, Y. P. Tang, Z. Q. Teng and C. Chen, *Cell Rep.*, 2012, **2**, 1329–1339.
55 C. Costola-de-Souza, A. Ribeiro, V. Ferraz-de-Paula, A. S. Calefi, T. P. Aloia, J. A. Gimenes-Junior, V. I. de Almeida, M. L. Pinheiro and J. Palermo-Neto, *PLoS One*, 2013, **8**, e77706.
56 S. G. Kinsey, L. E. Wise, D. Ramesh, R. Abdullah, D. E. Selley, B. F. Cravatt and A. H. Lichtman, *J. Pharmacol. Exp. Ther.*, 2013, **345**, 492–501.
57 J. W. Chang, M. J. Niphakis, K. M. Lum, A. B. Cognetta, 3rd, C. Wang, M. L. Matthews, S. Niessen, M. W. Buczynski, L. H. Parsons and B. F. Cravatt, *Chem. Biol.*, 2012, **19**, 579–588.
58 M. J. Niphakis, A. B. Cognetta, 3rd, J. W. Chang, M. W. Buczynski, L. H. Parsons, F. Byrne, J. J. Burston, V. Chapman and B. F. Cravatt, *ACS Chem. Neurosci.*, 2013, **4**, 1322–1332.
59 J. W. Chang, A. B. Cognetta, 3rd, M. J. Niphakis and B. F. Cravatt, *ACS Chem. Biol.*, 2013, **19**, 1590–1599.
60 S. G. Kinsey, J. Z. Long, S. T. O'Neal, R. A. Abdullah, J. L. Poklis, D. L. Boger, B. F. Cravatt and A. H. Lichtman, *J. Pharmacol. Exp. Ther.*, 2009, **330**, 902–910.
61 J. Guindon, A. Guijarro, D. Piomelli and A. G. Hohmann, *Br. J. Pharmacol.*, 2011, **163**, 1464–1478.
62 M. Alhouayek, D. M. Lambert, N. M. Delzenne, P. D. Cani and G. G. Muccioli, *FASEB J.*, 2011, **25**, 2711–2721.
63 S. G. Kinsey, S. T. O'Neal, J. Z. Long, B. F. Cravatt and A. H. Lichtman, *Pharmacol., Biochem. Behav.*, 2011, **98**, 21–27.
64 N. R. Sciolino, W. Zhou and A. G. Hohmann, *Pharmacol. Res.*, 2011, **64**, 226–234.
65 J. J. Sumislawski, T. S. Ramikie and S. Patel, *Neuropsychopharmacology*, 2011, **36**, 2750–2761.
66 D. Ramesh, G. R. Ross, J. E. Schlosburg, R. A. Owens, R. A. Abdullah, S. G. Kinsey, J. Z. Long, D. K. Nomura, L. J. Sim-Selley, B. F. Cravatt, H. I. Akbarali and A. H. Lichtman, *J. Pharmacol. Exp. Ther.*, 2011, **339**, 173–185.
67 D. K. Nomura, D. P. Lombardi, J. W. Chang, S. Niessen, A. M. Ward, J. Z. Long, H. H. Hoover and B. F. Cravatt, *Chem. Biol.*, 2011, **18**, 846–856.
68 D. K. Nomura, J. Z. Long, S. Niessen, H. S. Hoover, S. W. Ng and B. F. Cravatt, *Cell*, 2010, **140**, 49–61.
69 L. Ye, B. Zhang, E. G. Seviour, K. X. Tao, X. H. Liu, Y. Ling, J. Y. Chen and G. B. Wang, *Cancer Lett.*, 2011, **307**, 6–17.
70 I. A. Khasabova, A. Chandiramani, C. Harding-Rose, D. A. Simone and V. S. Seybold, *Pharmacol. Res.*, 2011, **64**, 60–67.
71 M. A. Sticht, J. Z. Long, E. M. Rock, C. L. Limebeer, R. Mechoulam, B. F. Cravatt and L. A. Parker, *Br. J. Pharmacol.*, 2012, **165**, 2425–2435.
72 K. L. Hsu, K. Tsuboi, A. Adibekian, H. Pugh, K. Masuda and B. F. Cravatt, *Nat. Chem. Biol.*, 2012, **8**, 999–1007.
73 K. L. Hsu, K. Tsuboi, J. W. Chang, L. R. Whitby, A. E. Speers, H. Pugh and B. F. Cravatt, *J. Med. Chem.*, 2013, **56**, 8270–8279.
74 V. Di Marzo, *Drug Discovery Today*, 2008, **13**, 1026–1041.
75 R. Christensen, P. K. Kristensen, E. M. Bartels, H. Bliddal and A. Astrup, *Lancet*, 2007, **370**, 1706–1713.
76 J. Z. Long, D. K. Nomura, R. E. Vann, D. M. Walentiny, L. Booker, X. Jin, J. J. Burston, L. J. Sim-Selley, A. H. Lichtman, J. L. Wiley and B. F. Cravatt, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 20270–20275.