Monoacylglycerol lipase inhibitors: modulators for lipid metabolism in cancer malignancy, neurological and metabolic disorders

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Abstract
Monoacylglycerol lipase (MAGL) is a serine hydrolase that plays a crucial role catalysing the hydrolysis of monoglycerides into glycerol and fatty acids. It links the endocannabinoid and eicosanoid systems together by degradation of the abundant endocannabinoid 2-arachidonoylglycerol into arachidonic acid, the precursor of prostaglandins and other inflammatory mediators. MAGL inhibitors have been considered as important agents in many therapeutic fields, including anti-nociceptive, anxiolytic, anti-inflammatory, and even anti-cancer. Currently, ABX-1431, a first-in-class inhibitor of MAGL, is entering clinical phase 2 studies for neurological disorders and other diseases. This review summarizes the diverse (patho)physiological roles of MAGL and will provide an overview on the development of MAGL inhibitors. Although a large number of MAGL inhibitors have been reported, novel inhibitors are still required, particularly reversible ones.

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1. Introduction

Monoacylglycerol lipase (MAGL) activity was initially discovered to hydrolyse monoglycerides (MAGs) into glycerol in the intestine and adipose tissue of rats\(^1,2\). Subsequently, the purification, cloning and enzymatic characterization of MAGL have involved many research groups\(^3,4\). MAGL, \(~33\) kDa serine hydrolase, contains an \(\alpha/\beta\) hydrolase fold and a catalytic triad with the nucleophilic serine in the highly conserved pentapeptide sequence Gly-X-Ser-X-Gly (GXSXG) found in lipases, where \(X\) represents any amino acid\(^4\). After around 40 years, MAGL was discovered to hydrolyse 2-arachidonoyl glycerol (2-AG) into arachidonic acid (AA) by Dinh et al\(^5\). 2-AG is an important endogenous signalling lipid that activates the cannabinoid receptors (CB\(_1\)R and CB\(_2\)R) and also serves as an important lipid precursor for the eicosanoid signalling pathway (Fig. 1a). Anandamide (AEA) is another main endogenous ligand for CB\(_1\)R and CB\(_2\)R. Both 2-AG and AEA, derivatives of AA, share some features of classical neurotransmitters but differ in others, for example, being produced on-demand rather than stored in vesicles\(^5\). 2-AG and AEA are produced and degraded by specific enzymatic pathways. For instance, the hydrolysis of 2-AG is mainly by MAGL, whereas AEA is hydrolysed mainly by fatty acid amide hydrolase (FAAH)\(^6,7\). AA, the metabolite of 2-AG and AEA, is the major precursor for pro-inflammatory prostaglandin synthesis. Since physiological 2-AG levels are much higher than those of AEA\(^2\), MAGL generated more interest and was brought back to the spotlight of research again. Thereby, several selective inhibitors and genetic mouse models of MAGL were developed to study the (patho)physiological roles of MAGL. Using these powerful tools, Nomura et al.\(^8\) demonstrated that MAGL was the major enzyme that provides AA for eicosanoid biosynthesis in certain tissues. Furthermore, many studies, both genetic and pharmacologic, have demonstrated the important roles of MAGL in the regulation of endocannabinoid and eicosanoid signalling pathways\(^9\). Thus, MAGL is considered as a promising therapeutic target for the treatment of various disorders, including neurodegenerative\(^12\), inflammation\(^13\)–\(^15\), metabolic diseases\(^16,17\) and even cancer\(^18\).

MAGL participates in the final lipolytic step of triacylglycerol catabolism and can broadly hydrolyse MAGs with different fatty acid chain length and saturation (e.g., 2-arachidonoyl glycerol, 2-palmitoylglycerol, 2-stearoylglycerol and 2-oleylglycerol)\(^19\). Among them, the degradation of 2-AG by MAGL has been studied extensively\(^7\). Since 2-AG is one of the most abundant endocannabinoids, it plays an essential role in the regulation of many physiological processes, including inflammation\(^20\), pain sensation\(^21\), neuroprotection\(^22\), food intake\(^23\), and addiction\(^24\). Although 2-AG can be metabolized by multiple enzymes, MAGL is still the

![Figure 1](image-url)  Signalling pathways regulated by monoacylglycerol lipase (MAGL) and their potential therapeutic roles. (a) MAGL inhibition induces an accumulation of the endocannabinoid 2-AG, which further enhances cannabinoid signalling by activation of CB\(_1\)R and CB\(_2\)R. MAGL modulates the production of the primary AA precursor pool for pro-inflammatory prostaglandins in tissues including brain, liver and lung. Thus, inactivation of MAGL has a variety of beneficial effects either by reducing eicosanoid production or enhancing endocannabinoid signalling; (b) MAGL also plays an important role in cancer cells by controlling FFA levels, which serves as sources for pro-tumorigenic signalling lipids (e.g., PGE2, lysophosphatidic acid) synthesis. MAGL inhibition reduced FFA production and attenuated cancer cell pathogenicity in aggressive cancer cells.
predominant one via hydrolysing the ester bond into AA and glycerol (Fig. 1a). AA acts as a substrate for several enzymes such as cyclooxygenases (COX-1 and COX-2), and can be further converted into inflammatory prostaglandins (PGs) and thromboxanes (Fig. 1a)25,26. For 2-AG biosynthesis, there are several proposed pathways. However, diacylglycerol lipases (DAGLs, containing two isoforms DAGLa and DAGLb) are considered as the important enzymes for 2-AG production through the hydrolysis of diacylglycerol (DAG, Fig. 1a)11. In addition, studies have shown that Magl-deficient mice have dramatically increased 2-AG levels in brain and peripheral tissues11,18. However, these increased 2-AG levels did not induce the cannabimimetic effects that can be observed in the mice with acute inhibitor treatment. This discrepancy may be due to the desensitization of CB1R in the brain, which is caused by the chronic elevation of 2-AG in Magl-deficient mice11.

In 2010, Nomura et al.18 found that MAGL was highly expressed in aggressive human cancer cells and primary tumours, where it regulates an oncogenic signalling network of lipids that promotes cancer cell migration, invasion, survival and tumour growth (Fig. 1b). Both pharmacological and genetical blockade of MAGL have induced significant elevations in MAGs and reductions in free fatty acids (FFAs) in aggressive cancer cells (Fig. 1b)18. These alterations of FFAs mediated by MGAL are only observed in aggressive human cancer cells, but not normal tissue, where MAGL mainly controls the levels of MAGs, but not of FFAs18,27. These results indicated that the MAGL–FFA pathway is essential in aggressive human cancer cells and primary tumours18. Additionally, the secondary lipid metabolites in the MAGL–FFA network can also be altered by suppression of MAGL. For example, the known oncogenic lipids including lysophospholipids (e.g., LPA, LPE and LPC), phosphatidic acid (PA), and prostaglandin (PGE2)28 have been reduced significantly by blockade of MAGL (Fig. 1b). These alterations contribute partially to the role of MAGL in cancer pathogenicity in aggressive human cancer cells. Of note, the exact pathways that FFAs are converted to oncogenic lipids are still unclear, although there are some proposed ones13,18. More studies about the role of MAGL in cancer cells will be discussed in detail in later section.

As summarized in Fig. 1, MAGL plays a critical role in lipid signalling: i) it is the major enzyme that controls the levels of 2-AG, an important lipid with various neuroprotective effects; ii) inactivation of MAGL induces an elevation in brain levels of 2-AG and a reduction of AA, a key precursor of pro-inflammatory prostaglandins, resulting in the reduction of neuroinflammation; iii) MAGL regulates the levels of FFAs in aggressive cancer cells, and this MAGL-promoted fatty acid network drives a number of pro-tumorigenic signalling pathways. Based on these, MAGL is emerging as a promising drug target for various diseases.

The determination of the crystal structure of MAGL provides the evidence how the enzyme interacts with substrate and inhibitors and gives insights to future MAGL inhibitor development29,30. Substantial efforts by research groups and pharmaceutical companies have led to the development of MAGL inhibitors. In general, two types of inhibitors have been reported based on the reaction mechanisms: i) inhibitors that covalently and irreversibly bind on MAGL; ii) inhibitors that bind reversibly to MAGL31,32. Among them, irreversible inhibitors are the majority for MAGL, and only a few reversible inhibitors have been reported recently. Here, we focus on an overview of MAGL, including its structural features and biochemical properties, tissue distribution and (patho)physiological roles, the current state of MAGL inhibitor development and their therapeutic potential.

2. Structure, distribution, biochemistry and physiology of MAGL

2.1. Structural features and biochemical properties

MAGL is a membrane-associated soluble enzyme, which belongs to the serine hydrolase superfamily. MAGL was first purified from rat adipose tissue in 197619 and cloned from mouse adipocytes in 199717. The X-ray crystal structure of MAGL was reported in 200919. MAGL crystallizes as a dimer and belongs to the α/β hydrolase superfamily. MAGL contains the cap domain in the structure, which is substantially different from that of other proteins in the superfamily (Fig. 2). Crystallographic data have revealed that the cap is flexible, indicating the existence of other conformations. Ser122-Asp239-His269 residues were identified as the catalytic triad for MAGL, with Ser122 identified as the nucleophile interacting with the carbonyl group of the substrate (Fig. 2). There is a wide hydrophobic entry to the catalytic site of MAGL, with the entry edge nearby hydrophobic helices, which suggests the amphiphilic character of MAGL and likely allows MAGL to interact with membranes and recruit lipophilic substrates. Additionally, three cysteines (Cys201, Cys208, and Cys242) located near the catalytic triad were proposed to stabilize the active conformation of MAGL19. These cysteine residues also provide opportunities to develop selective MAGL inhibitors over other serine hydrolases.

MAGL preferentially hydrolyses monoacylglycerols to glycerol and fatty acids with no positional preference for sn-1 (3) or 2-monoacylglycerols (MAGs)19,35. MAGs are always short-lived lipids, which could be from both intra- and extracellular. One of the important MAGs is endocannabinoid 2-AG, which can be degraded into arachidonic acid and glycerol36. In most tissues including brain, more than 80% of 2-AG hydrolytic activity is prevented by inhibition of MAGL, this suggests the dominant role of MAGL for 2-AG degradation37,38. FAAH, the key enzyme for degradation of AEA, has also been reported to contribute to the degradation of 2-AG to some extent in vitro19,35. Other studies have indicated that prostaglandin glycerol esters, the poorly characterized inflammatory mediators, could also be hydrolysed by MAGL35. More recently, MAGL has been identified to hydrolyse fatty acid ethyl esters that are generated in response to alcohol consumption41.

2.2. Tissue distribution and physiological roles of MAGL

MAGL is highly expressed in brain, liver, adipose tissue, intestine, and others, and that have been demonstrated by both genetic and pharmacological inhibition of MAGL in mice. In brain, MAGL is expressed in neurons, astrocytes, and oligodendrocytes, and in microglia (to a lower extent)41,42. Western blot studies revealed heterogeneity of MAGL protein of multiple molecular weights19,35. In mice, a single MAGL band is observed at ~33 kDa in adipose tissue, liver, lung, heart, kidney, spleen, and adrenal glands, whereas in brain, testis, and skeletal muscle, MAGL migrates with another molecular weight19. In brain, MAGL bands were observed at ~33 and 35 kDa. In testis, MAGL migrates as a single band of ~30 kDa. In muscle, MAGL migrated at a molecular weight of
such as activity was reported to be responsible by other serine hydrolases, activity by at least 80%. The remaining pharmacological inhibition of MAGL reduces 2-AG hydrolytic selective MAGL inhibitors that are active MAGL has recently been accelerated by the development of se-

splicing, which would meet specific needs of a cell in the particular tissue of physiological process. The understanding of the metabolic and physiological roles of MAGL has recently been accelerated by the development of selective MAGL inhibitors that are active in vivo such as JZL184, and of Magl knock out mice (Magl<sup>-/-</sup>). In brain, genetic or pharmacological inhibition of MAGL reduces 2-AG hydrolytic activity by at least 80%. The remaining ~ 20% of 2-AG hydrolytic activity was reported to be responsible by other serine hydrolases, such as a/b-hydrolases 6 and 12 (ABHD6 and ABHD12) ABHD6 and ABHD12 contribute to approximately 20% of 2-AG hydrolysis, however, the exact roles in 2-AG metabolism and signalling are still unclear. MAGL is the primary enzyme responsible for 2-AG degradation, which is confirmed by MAGL inhibitors and Magl<sup>-/-</sup> mice models. Recent reports suggest that ABHD6 acts as the dominant enzyme for 2-AG hydrolysis in cells where MAGL is not expressed. In neurons, both ABHD6 and MAGL are expressed but with different subcellular distribution. ABHD6 is localized at post-synaptic membranes and MAGL is predominantly observed presynaptically. ABHD6 is suggested to contribute 2-AG formation at the post-synaptic site, while MAGL is mainly for pre-synaptic site. In peripheral tissues, inhibition of MAGL by JZL184, a known potent and selective MAGL inhibitor, led to the accumulation of 2-AG to varying extents. In testis and adipose tissue, there was 40%–50% reduction of 2-AG hydrolysing activity after JZL184 treatment, which suggests the presence of another hydrolase in these tissues, or the potential impact of alternative species on MAGL activity. Of note, the complete inhibition of MAGL activity by JZL184 in these tissues is considered.

In addition to the reduction of monoacylglycerol levels such as the major AA-releasing enzyme in gut, spleen and macrophages, whereas MAGL plays the dominant role to produce AA in brain, liver and lung. Magl-deficient mice or chronic pharmacological inhibition of MAGL leads to partial desensitization of the CB<sub>1</sub>R in the brain and loss of cannabinoid-mediated effects and produces cross-tolerance to exogenous CB<sub>1</sub> agonists due to functional antagonism. Additionally, Magl<sup>-/-</sup> mice also have impaired CB<sub>1</sub>-dependent synaptic plasticity and physical dependence. Therefore, it worth of interest to find an inhibition window for MAGL that maintains endocannabinoid signalling under chronic inhibition.

3. MAGL and diseases

3.1. MAGL in inflammation and neurological disorders

Cannabinoids have been used as analgesics for a quite long time, and only recently endocannabinoid system has been linked to inflammation. Inflammatory processes are always associated with multiple neurodegenerative disorders. Moreover, pain and inflammatory processes are considered to be a hallmark of neurological diseases, including Alzheimer’s disease (AD), Parkinson’s disease (PD), multiple sclerosis (MS) and stroke. CB<sub>1</sub>R and CB<sub>2</sub>R agonists and cyclooxygenase (COX) inhibitors have been shown to have beneficial effects on various inflammatory diseases. However, use of COX1 and COX2 inhibitors have been limited because they can cause gastrointestinal and cardiovascular injury. MAGL has been discovered to reduce AA and prostaglandins levels in specific tissues, suggesting its potential as a therapeutic target for inflammation. In LPS-treated mice, administration of a MAGL inhibitor reduced pro-inflammatory prostaglandin and cytokine formation. MAGL inhibition has produced neuroprotective effects in animal models of Parkinson disease and multiple sclerosis. MAGL inhibition leads to accumulation of 2-AG and activation of cannabinoid receptors; however, these neuroprotective responses seem not to be driven via cannabinoid receptor dependent pathway, but lowering pro-inflammatory eicosanoids. The attenuated neuroinflammatory responses in animal models were not reversed upon cannabinoid receptor antagonists, indicating that the observed protective effects were mainly due to the reduction of prostaglandin and...
cytokine levels in brain. However, chronic MAGL inhibition that induces the functional desensitization of the cannabinoid system might also contribute to the neuroprotective response.

3.2. MAGL in metabolic disorders

Metabolic disorders are a major public health care concern that has been associated with the endocannabinoid signalling. CB1R activation induced the increase of lipogenesis and lipid deposition, hyperphagy and hypomotility56. In contrast, Ch141 mice showed hypophagia, leanness, hepatic steatosis and insulin resistance56,57. Blockade of CB1R by the selective inverse agonist rimonabant reduced hepatic steatosis and dyslipidemia in animal models. Rimonabant (Acomplia60), a promising drug for obesity treatment, could reduce weight loss and improve cardiovascular risk factors, but it had to be removed from the market because of severe central psychiatric side effects58. Magl−/− mice were recently reported to have reduced body weight upon both low-fat diet (LFD) and high-fat diet (HFD). Besides, Magl−/− mice were found significantly leaner than WT mice, the serum lipid levels in Magl−/− mice were decreased as well59. Additionally, in pancreatic β-cells, MAGL was discovered to regulate insulin release and recent data also showed that glucose-stimulated and depolarization-induced insulin secretion were prevented by MAGL inhibitors59. Taken together, these data suggest that selective inhibition of MAGL might represent a new alternative therapeutic avenue to treat metabolic disorders.

3.3. MAGL in cancer

Beyond inflammation and metabolic disorders, MAGL was also implicated to play a pathophysiological role in cancer. Nomura et al.18 demonstrated that MAGL activity was highly elevated in multiple types of aggressive human cancer cells, including ovarian, breast and melanoma cancer cells. MAGL was discovered to be involved in several cellular processes in these cells, including cellular growth, survival, migration and invasion. These studies suggest that MAGL promotes cancer aggressiveness by providing a pool of FFAs for oncogenic signalling of lipid synthesis. Inhibition of MAGL induced the reduction of FFAs, lysophosphatidic acid and prostaglandins, leading to the decrease of cancer cell aggressiveness, reportedly independent on endocannabinoid signalling18. Nomura et al.50 further reported the high activity of MAGL in prostate cancer cells, and inhibition of MAGL activity impaired prostate cancer aggressiveness through FFAs reduction and CB1R activation. Other studies have demonstrated the high expression of MAGL in colorectal cancerous tissues, and tumorigenesis in colorectal cancer cell lines was impaired by MAGL inactivation57. Recently, a high expression of MAGL was also detected in nasopharyngeal and hepatocellular carcinoma, and knockdown of these cells reduced cellular migration62,63. Additionally, MAGL inhibition has been observed to have effects on cancer-associated symptoms, including alleviating pain and quelling nausea59. These studies suggest that MAGL plays a distinct role in driving cancer malignancy and is a potential therapeutic target for cancer treatment. However, more research is still required to explore the role of MAGL in malignant human cancer cells, for example, to determine whether the mechanism is cannabinoid signalling dependent or independent. In addition, inhibitors of MAGL have shown promise as anti-cancer agents, while alleviating cancer-associated symptoms, and may contribute to the understanding of the physiological role of MAGL in cancer aggressiveness.

4. Assays to measure MAGL activity

4.1. Surrogate substrate assay

Several types of MAGL activity assays are currently available. The first type of assay employs surrogate substrates, for example, 4-nitrophenylacetate (4-NPA)65 and 7-hydroxycoumarinyl arachidonate (7-HCA)66, which mimics the reaction between MAGL and its natural substrate 2-AG (Fig. 3a). A surrogate substrate assay is generally used for inhibitor identification due to its cost-effectiveness and product easy detection. Surrogate substrate assays have multiple advantages. For example, enzymatic reaction progress can be monitored real-time by measuring absorption or fluorescence (Table 1). According to the product detection methods, radiometric assays have also been used to detect MAGL activity in vitro, utilizing radiolabelled substrate, such as [3H]-2-oleoylglycerol ([3H]-2-OG)67,68. This method is more sensitive than previous methods using absorption or fluorescence detection, however, the wide-spread use of radiometric assays is limited by complex experimental procedures, including lipid extraction, fractionation on thin layer chromatography, and radiolabelled substrate ([3H]-2-OG) quantification. With the development of the 4-NPA-based surrogate substrate assay, Sanofi–Aventis68 identified a highly potent and selective MAGL inhibitor SAR127303. However, to some extent, surrogate substrate assays are limited in their ability to evaluate inhibitor activities, and also may affect the determination of inhibitor potency (e.g., IC50 values) with artificial substrates (Table 1). For example, binding affinities with MAGL usually have been attenuated compared with MAGL's natural substrate 2-AG. For this reason, additional assays are required to confirm the potency of inhibitors, for example, natural substrate assays are always preferred for inhibitor potency confirmation.

4.2. Natural substrate assay

Several classes of natural substrate assays exist to measure MAGL activity. Liquid chromatographic methods coupled with mass spectrometry (LC–MS) have been used to directly measure AA formation69,70. For example, Takeda70 has identified piperazinyl pyrrolidin-2-ones as a novel series of reversible MAGL inhibitors based on this type of assay. LC–MS-based assays are highly sensitive and accurate, but require lipid extraction and phase separation. LC–MS-based assays are more costly and less high throughput compared with surrogate substrate assays (Table 1). Therefore, LC–MS-based assays are not ideal for inhibitor screening. Furthermore, LC–MS-based assays cannot monitor an enzymatic reaction progress in real-time, because of the discontinuous setup. Besides, a coupled enzyme glycerol assay for MAGL has been developed based on an enzymatic cascade reaction that couples the conversion of the natural substrate 2-AG to the formation of a fluorescent signal (Fig. 3b)71. This assay allows 2-AG hydrolysis to be studied in real-time in 96-well (or even 384-well) plates using recombinant MAGL and avoiding the lipid extraction step. Natural substrate assays are generally used to further confirm inhibitor potency, and other more reliable results may be obtained from them compared with surrogate substrate assays.
4.3. Activity-based protein profiling (ABPP)

Recently, competitive ABPP was employed as another class of assay to identify and optimize inhibitors for multiple enzymes (Fig. 4). ABPP is a powerful and robust chemical biology technique to study target engagement of inhibitors in a native system. Competitive ABPP makes use of activity-based probes (ABPs) that label the active sites of target enzymes from lysates, intact cells or even animal tissues to assess the activity and selectivity of inhibitors in a single experiment without the need of having substrate (Table 1)\textsuperscript{72,73}. In competitive ABPP, inhibitors are pre-incubated with a biological sample, and enzyme activities are subsequently detected and monitored by ABP (Fig. 4). Broad-spectrum ABPs that target a whole (or to a large extent) family of proteins, are generally used for inhibitor identification and optimization\textsuperscript{72}. ABP generally consists of a warhead, a linker and a reporter tag (e.g., fluorophore, biotin, Fig. 4a). Fluorescent tags are usually used for gel-based ABPP assays, and biotin tags are used to identify and characterize the interacting proteins by mass spectrometry\textsuperscript{72}. Competitive ABPP assays are able to assess activity and selectivity of inhibitors both in vitro and in vivo, and are highly valuable and complementary method to traditional substrate assays\textsuperscript{72}. Currently, two structurally different ABPs have been developed for detection of MAGL activity in proteomes:

Table 1  A summarization of the pros and cons for surrogate substrate assays, natural substrate assays and ABPP assays.

| Assay                        | Pros                                                                 | Cons                                                                 |
|------------------------------|----------------------------------------------------------------------|----------------------------------------------------------------------|
| Surrogate substrate assays   | Cost-effectiveness; easy detection of the product; enzymatic reaction progress can be monitored in real-time. | Binding affinities of enzymes can be attenuated due to artificial substrate; inhibitor potency (e.g., IC\textsubscript{50} values) might be affected by use of different surrogate substrates. |
| LC–MS-based assays (natural substrate assays) | Highly sensitive and accurate.                                        | Costly; less high throughput; cannot monitor enzymatic reaction progress in real-time; complex experimental procedures (e.g., lipid extraction); limited samples can be acquired and measured. |
| Fluorometric glycerol assay (natural substrate assays) | Using natural substrate (2-AG); enzyme inhibition can be tested in a more physiological condition; enzymatic reaction progress can be monitored in real-time; application in high throughput screening. | False-positive reduction: compounds interacting with glycerol should be excluded; experimental procedure is less straightforward. |
| ABPP                         | Without the need of substrate; activity and selectivity can be measured in one single experiment; both in vitro and in vivo activity/selectivity can be measured; a selectivity profile across entire proteome can be measured. | An effective activity-based probe is required; gel-based ABPP assay is less high throughput. |
5. MAGL inhibitors

According to the interactive mechanism between inhibitors and enzyme, the inhibitors can generally be divided into two classes: reversible and irreversible (Fig. 5)\(^7\). Initially, the inhibitor binds to the enzyme—enzyme—inhibitor (EI) complex. Subsequently, the EI complex can either i) reverse to release the free enzyme (reversible inhibitor), or ii) undergo a tightening interaction. For reversible inhibitors, enzyme activity is recovered due to the non-covalent association and the reversible equilibrium with the enzyme (Fig. 5a)\(^77\). For irreversible inhibitors, the complex shifts from EI to form EI\(_{\text{inact}}\) preventing the dissociation of EI (Fig. 5b)\(^77\). However, some inhibitors undergo a conversion from EI\(_{\text{inact}}\) to the transition state EI, and are defined as partially/slowly reversible inhibitors\(^76,79\) (Fig. 5c)\(^77\). To reverse the partially reversible inhibitor—enzyme complex EI\(_{\text{inact}}\) special treatments such as dialysis are required\(^76,77\).

To date, a couple of chemotypes have been reported as MAGL inhibitors, which can be classified into (a) irreversible inhibitors: maleimides, disulfides, carbamates, ureas and arylthiocarbamides, and (b) reversible inhibitors: tetrahydroplastatin-based derivatives (β-lactones), isothiazolines, natural terpenoids and amide-based derivatives, etc. Among them, the most reported MAGL inhibitors are irreversible ones, whereas only recently have reversible inhibitors been reported.

5.1. Irreversible inhibitors

5.1.1. Maleimides

Unlike other serine hydrolase, MAGL has been demonstrated to interact with sulphydryl-sensitive inhibitors, mostly due to the active cysteine residues near its active site (Cys201, Cys208 and Cys242)\(^77\). Maleimide derivatives such as N-ethylmaleimide (1) (Fig. 6a), comprising mercapto-specific functions, were identified as the starting points for MAGL inhibitor development. Structure—activity relationship (SAR) modification by increasing lipophilicity resulted in the identification of N-arachidonoyl maleimide (NAM, 2) with an IC\(_{50}\) of 1.12 μmol/L against human MAGL\(^76\) (Fig. 6a and c, Table 2\(^79,84,88,90,93,95\) ). Further modification of NAM led to the discovery of compound 3 (Fig. 6a), which showed higher activity (IC\(_{50}\) = 0.79 μmol/L) and better selectivity against human MAGL over FAAH in comparison with NAM\(^76\). Maleimide derivatives are proposed to interact covalently and irreversibly with the sulphydryl group of cysteine residues through a Michael addition to form a S-alkylated MAGL adduct (Fig. 6b)\(^76\). Although NAM and 3 are active and selective against MAGL, they have been rarely used for functional studies because the maleimide group, which is considered as a thiol-reactive electrophile, might react with other cysteine-containing proteins or enzymes.

5.1.2. Disulfides

Disulfiram (4), an aldehyde dehydrogenase (ALDH) inhibitor used to treat alcoholism, was reported to show inhibitory activity against human MAGL with a pIC\(_{50}\) of 5.90 (IC\(_{50}\) = 0.36 μmol/L)\(^7\) (Fig. 7 and Table 2). Based on the scaffold of 4, a series of analogues with different N-substitutions have been synthesized to improve the activity and selectivity for human MAGL. To the end, compounds 5 and 6 were identified to exhibit optimal activity against human MAGL with pIC\(_{50}\) of 6.96 and 6.78 μmol/L, respectively (Fig. 7)\(^81\). Both of these compounds showed more than 1000-fold selectivity over human FAAH, particularly, compound 6 rarely inhibited human FAAH even at 1 μmol/L concentration (Fig. 7b)\(^81\). According to the exploration of the reversibility, disulfide derivatives were presumed to irreversibly interact with MAGL by formation of disulfide bonds with MAGL cysteine residues (Cys208 and Cys242).

5.1.3. Carbamates

URB602\(^82\) (Fig. 8), the first-generation of MAGL O-aryl carbamate inhibitor, modestly inactivated rat brain MAGL with an IC\(_{50}\) of 28 μmol/L (Table 2). URB602 showed low potency in vivo, and increased (∼2-fold) 2-AG concentrations, but had no effect on AEA. However, the relative low potency against MAGL and cross-reactivity with FAAH\(^84\) make URB602 unsuitable to study the physiological role of MAGL. To determine the reversibility of URB602, dialysis experiments were performed, suggesting that URB602 is not a full irreversible agent, but partially reversibly binds to MAGL\(^86\). It was also proposed that an O-substituent of URB602 was the leaving group after hydrolysis.

In 2009, Long et al.\(^7\) identified the first selective and in vivo active MAGL inhibitor JZL184 (8) (Fig. 8) by using the robust technique ABPP (Fig. 4). Discovery of JZL184 is considered as one of the important breakthroughs for MAGL inhibitor development, and it accelerated our understanding of MAGL’s physiological roles. JZL184, a piperidine carbamate, is regarded to inhibit enzyme activity covalently and irreversibly by carbamylating a serine residue in the active-site of MAGL. JZL184\(^8\) shows high inhibitory potency against MAGL at nanomolar range (IC\(_{50}\) = 8 nmol/L, mMAGL) (Table 2). Competitive ABPP indicated that JZL184 was around 100-fold more selective for MAGL over FAAH and other serine hydrolases in mouse brain. In peripheral tissues, however, JZL184 exhibited inhibitory effects on several other enzymes, including esterase 1, esterase 1-like, and triacylglycerol hydrolase\(^27\). In vivo experiments showed that 2-AG hydrolysis was inhibited ∼85% in mouse brain by inhibition of MAGL with JZL184, resulting in a dramatic increase of 2-AG levels in brain\(^8\). JZL184 exerted a long duration of action, and MAGL inhibition was up to 24 h. The maximal elevation of 2-AG induced by JZL184 (i.p. 16 mg/kg, single dose) lasted for 8 h\(^9\). Beneficial effects were observed by the administration of JZL184 in multiple animal models, including pain alleviation, inflammation, emesis, anxiety, neurodegeneration, and cancer pathogenicity\(^11,95,96\). JZL184 serves as an important chemical tool and drug candidate to pharmacologically explore the (patho)physiological roles of MAGL. However, subsequent studies have
disclosed that chronic and complete inhibition of MAGL by JZL184 induced desensitization of CB1R in mouse brain. CB1R desensitization is a loss of cannabinoid-mediated effects and physical dependency, that is also observed in Magl knockout mice. This might be due to the complete inhibition of MAGL, since further studies revealed that chronic and partial inhibition of MAGL maintained the CB1-mediated signalling, and avoided the functional antagonism of the cannabinoid system. Of note, although JZL184 is a highly potent MAGL inhibitor, it also cross-reacts with several other off-targets such as ABHD6. Thus, further structural modification of JZL184 was continued, resulting in the generation of several new carbamate derivatives, including the O-hexafluoroisopropyl (HFIP) and N-hydroxysuccinimidyl (NHS) analogues (compounds 9–11; Fig. 8 and Table 2). Among them, KML29 (9), a derivative of JZL184 with HFIP as a leaving group, displayed a complete selectivity for MAGL over FAAH in ABPP assays both in vitro and in vivo. Notably, KML29 was selective over ABHD6 to some extent (only observed ABHD6 inhibition at 10 µmol/L), comparing with JZL184 and other derivatives. Moreover, unlike JZL184, KML29 did not show any inhibition of carboxylesterases (e.g., esterase 1 and esterase 1-like) even at high doses (40 mg/kg) in mouse liver, and only exhibited minimal inhibition against carboxylesterase 1 in mouse lung. Of note, JZL184 shows little inhibition of rat MAGL both in vitro and in vivo, however, KML29 maintains high potency and selectivity against rat MAGL and increases 2-AG

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**Figure 4** Overview of activity-based protein profiling (ABPP). (a) Representative cartoon structure of activity-based probe (ABP): reactive group (blue), linker (grey), and reporter tag (red); (b) in competitive ABPP, proteomes (tissue or cell lysates) are pre-incubated with inhibitors, and ABPs (broad-spectrum direct probes) are added subsequently; (c) click chemistry ABPP (CC-ABPP) provides a direct measurement of probe labelling events, and a more global map of covalent interaction.

**Figure 5** Binding mechanism of reversible, irreversible and partially reversible inhibitors. (a) Reversible inhibitors interact with the enzyme to form a transition state complex (EI) in a reversible way; (b) irreversible inhibitors initially bind to the enzyme to form the EI complex and subsequently irreversibly inactivate the enzyme, generating a permanent inactive form Ei_{inactive}, which prevents the dissociation of the EI complex (K_a > 0). (c) Partially reversible inhibitors undergo a conversion from Ei_{inactive} to the transition state EI (K_a > 0, K_b > 0).
levels in rat brain. As a carbamate-based inhibitor, KML29 was demonstrated to covalently and irreversibly react with MAGL by the formation of the carbamylated enzyme— inhibitor adduct. A subsequent modification of KML29 on the staying group led to the discovery of JW651, which maintains similar activity but with improved selectivity for MAGL, compared with JZL184 and KML29. JW651 inhibited human MAGL at 100 nmol/L in vitro and complete inhibited MAGL in mouse brain at doses as low as 5 mg/kg. JW651 was highly selective against MAGL even at high concentration (100 µmol/L) in vitro and high dose (40 mg/kg) in vivo, with ABHD6 the only identified off-target inhibition in mouse brain. Later on, Niphakis et al. reported MJN110, a carbamate-based inhibitor with the replacement of the HFIP group in JW651 by a NHS group. MJN110 exhibited high potency and selectivity against MAGL. MJN110 was able to completely inhibit MAGL at 1 µmol/L and in vivo inhibition was observed as low as 1 mg/kg (oral or intraperitoneal injection). To comprehensively study the selectivity profiles of JW651 and MJN110 on a proteome-wide level, click chemistry ABPP was applied by using alkyne-bearing analogues of JW651 and MJN110 (clickable probes JW651yne and MJN110yne, Fig. 8). The results of click chemistry ABPP have confirmed that both of the inhibitors were selective across the entire proteome. Taken together, the discovery of highly potent, selective and irreversible carbamate-based MAGL inhibitors such as JW651 and MJN110 provides important tools for target validation, and these compounds also serve as useful templates for drug discovery.

In 2015, Sanofi reported a new class of carbamate-based inhibitor SAR127303 with little similarities to known MAGL inhibitors. SAR127303 showed high potency towards recombinant human MAGL with an IC\textsubscript{50} of 48 nmol/L in a biochemical assay with 4-nitrophenylacetate (4-NPA) as a substrate. In their study, a series of 3-substituted azetidine inhibitors, including five- and six-membered heterocycles, were rationalized as the ligand efficiency, correcting the potency values of a heavy atom. In their study, a series of 3-substituted azetidine analogues were discovered and retained high MAGL potency (IC\textsubscript{50} = 0.18 nmol/L, hMAGL), suggesting pyrazole could serve as an efficient linker of the azetidines. Continued optimization of the pyrazole substituent did not significantly improve the potency, but maximized LipE values of the inhibitors by substituting with pyrazine (compound 16). According to the in vitro data, compound 15 was selected as a well-optimized MAGL inhibitor, and the selectivity profile of 15 was evaluated by ABPP against a panel of 42 serine hydrolases. The results demonstrated that 15 significantly inhibited (>70%) ABHD6, CES1, CES2, MAGL and PLA2G7 at 1 µmol/L, and FAAH at 10 µmol/L. Additionally, the activity and proteome-wide selectivity in human brain vascular pericytes (cellular context) were determined by click chemistry ABPP with alkyne-containing probe 17. Clickable probe 17 was selective against MAGL at low concentration (<IC\textsubscript{50}), whereas an additional protein band at ~40 kDa molecular weight was observed at high concentrations from sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) results. In vivo experimental studies of 15 have shown to elevate central 2-AG levels at a dose of 10 mg/kg, implicating the in vivo inhibitory activity of 15. However, the increase of 2-AG levels in mouse brain by 15 was less robust comparing with the corresponding piperidine 18, suggesting the azetidine-derived analogues might exhibit reduced pharmacodynamics effects. The authors speculated that the azetidine adducts formed at the active site of MAGL might be rapidly hydrolysed from the enzyme, whereas compounds containing larger ring cores such as piperidine 18 might have improved MAGL adduct stability and produced prolonged pharmacodynamics effects. Thus, further effects were focused on the optimization of the core system of azetidine-derived inhibitors. To this end, compound 19, based on a [3.1.0] bicyclic core system, was discovered and retained high MAGL potency (IC\textsubscript{50} = 1.4 nmol/L) and excellent selectivity. Although compound 19 has a promising pharmacology profile, the high lipophilicity and poor kinetic solubility (<1 µmol/L) make it a less than ideal inhibitor. To address this, the leaving groups were systematically explored to improve drug-like properties, resulting in the identification of inhibitor PF-06795071 (20, IC\textsubscript{50} = 3 nmol/L), which features a novel stereodefined trifluoromethyl glycol leaving group. PF-06795071 has an excellent MAGL pharmacology profile, along with significantly improved physicochemical properties and LipE value. PF-06795071 also showed high selectivity over other serine inhibitors were selective across the entire proteome. The results of click chemistry ABPP have confirmed that both of the inhibitors were selective against MAGL. MJN110 was able to comprehensively study the selectivity profiles of JW651 and MA
hydrolases and a clean in vitro safety profile. Moreover, PF06795071 showed good central nervous system (CNS) distribution in mouse brain and a suitable pharmacokinetic (PK) profile, and could be used as an ideal tool to study in vivo efficacy. Administration of PF06795071 robustly increased mouse brain 2-AG accumulation, demonstrating the high in vivo efficacy of this compound. Notably, PF06795071 also reduced brain inflammatory markers in response to LPS challenge. The discovery of this glycol leaving group series provides an important scaffold for developing MAGL inhibitors, which can be used to treat neuroinflammatory conditions with injection of vein delivery. Most recently, Abide Therapeutics reported a carbamate-based irreversible inhibitor ABX-1431, which is a first-in-class experimental drug for MAGL. To the discovery, optimization and profiling of ABX-1431, ABPP is the key technology that has been used. Particularly, competitive ABPP (Fig. 4b) was applied to

| Chemotype | Inhibitor | Reversibility | MAGL inhibition (IC50 mmol/L) | Surrogate substrate assay | Natural substrate assay | ABPP assay |
|-----------|----------|---------------|-------------------------------|--------------------------|------------------------|------------|
| Maleimides | NAM (2) | Irreversible | 112086 (hMAGL) | | | |
| Disulfides | Disulfiram (4) | Irreversible | 36086 (hMAGL) | | | |
| | Compound 6 | Irreversible | 16686 (hMAGL) | | | |
| Carbamates | URB602 (7) | Partially reversible | 1000086 (hMAGL) | 2800086 (rMAGL) | | |
| | JZL184 (8) | Irreversible | 48086 (hMAGL) | | | |
| | KML29 (9) | Irreversible | 2.586 (mMAGL) | 1586 (mMAGL) | | |
| | | | 0.8586 (mMAGL) | 4386 (rMAGL) | | |
| | | | 2.586 (rMAGL) | 5.986 (hMAGL) | | |
| | | | 3.686 (hMAGL) | | | |
| | JW651 (10) | Irreversible | 4.586 (mMAGL) | 38.75 (mMAGL) | | |
| | MIN110 (11) | Irreversible | 2.186 (mMAGL) | 9.586 (mMAGL) | | |
| | SAR127303 (14) | Irreversible | 4886 (hMAGL) | | | |
| | PFO6795071 (20) | Irreversible | 386 (hMAGL) | | | |
| | ABX-1431 (21) | Irreversible | | | | |
| Ureas | SAR629 (22) | Irreversible | 0.2286 (mMAGL) | 1.186 (rMAGL) | | |
| | ML30 (23) | Irreversible | 0.5487 (hMAGL) | 1.986 (mMAGL) | | |
| | | | 4.486 (mMAGL) | 1.586 (hMAGL) | | |
| | JKKK-048 (25) | Irreversible | 0.2886 (mMAGL) | 0.2486 (mMAGL) | | |
| | | | 0.3686 (mMAGL) | | | |
| Arythioamides | CK37 (30) | Irreversible | 15486 (hMAGL) | | | |
| | OMDM169 (31) | Partial reversible (covalently) | 7.386 (mMAGL) | 0.3486 (mMAGL) | 89086 (hMAGL) | |
| | | | 9386 (rMAGL) | 31586 (rMAGL) | | |
| Natural terpenoids | Pristimerin (35) | Reversible | 9386 (rMAGL) | | | |
| | Euphol (36) | Reversible | 31586 (rMAGL) | | | |
| Isothiazolines | Octilitonone (32) | Partial reversible | 8814 (rMAGL) | 18086 (mMAGL) | 24086 (rMAGL) | |
| | Compound 41 | Reversible | 84086 (hMAGL) | 26286 (hMAGL) | | |
| | Compound 44 | Reversible | 8086 (hMAGL) | | | |
| | Compound (R)-49 | Reversible | 3.686 (hMAGL) | | | |

Note: mMAGL, mouse brain MAGL; rMAGL, rat brain MAGL; hMAGL, human recombinant MAGL.

Figure 7 Disulfide-based MAGL inhibitors. (a) Chemical structures of disulfide-based MAGL inhibitors 4–6; (b) activities of disulfide-based inhibitors 4–6 against human MAGL and FAAH.
study target engagement for covalent irreversible inhibitors in living systems. Initially, HFIP carbamate-based MAGL inhibitors such as KML29 and JW651 were chosen as staring points for the rational design of novel MAGL inhibitors. To optimize both MAGL potency and serine hydrolase selectivity simultaneously, Cisar et al. used competitive gel-based ABPP with two distinct activity-based probes (FP-Rh and JW912) for the initial screening analysis. ABPP probe JW912 provides initial potency and selectivity information for MAGL inhibitors in multiple human proteomes. Of note, the selectivity assessment by JW912 is only limited to off-targets ABHD6 and phospholipase A2 group VII (PLA2G7). Therefore, they used an additional ABPP probe FP-Rh, which enabled detection of many serine hydrolases, to evaluate the interaction of their MAGL inhibitors on the serine hydrolase family. The first round of optimization starts with a symmetrical biaryl analogue JW651 (10); however, the replacement of the phenyl group by various heterocyclic rings did not significantly improve the potency or selectivity. To reduce the high lipophilicity of JW651 analogues, the authors evaluated the impacts of aryl groups by removing one of them, which resulted in the improved selectivity profile, while maintaining high MAGL potency. Further systematic SAR studies on the phenyl substitutions led to the discovery of pyrrolidine analogue ABX-1431 (21), Fig. 8), which displayed high potency and selectivity for MAGL in human PC3 lysates and rodent MAGL brain homogenates. ABX-1431 (IC\textsubscript{50} = 8 nmol/L, recombinant human MAGL) is described as a highly selective, potent and CNS-penetrant MAGL inhibitor. Moreover, this compound has excellent drug-like property and is suitable for once-per-day oral administration. Mass spectrometry-based ABPP has demonstrated that ABX-1431 retains high activity and selectivity in human cellular assays and human prefrontal cortex proteomes. Although ABX-1431 is a lipophilic molecule and has a basic amine, it showed only weak hHEG channel activity (IC\textsubscript{20} ~ 7 μmol/L). Additionally, ABX-1431 did not display any significant activity against a panel of 95 common off-targets including enzymes, receptors, transporters,
and ion channels at 10 μmol/L, and had low propensity to inhibit human recombinant CYP (cytochrome P450 proteins) enzymes (IC₅₀ > 50 μmol/L for CYP1A2, CYP2C9, CYP2C19, CYP3A4/5; IC₅₀ = 6.5 μmol/L for CYP2D6). Furthermore, analysis in various transporter assays have shown that ABX-1431 was not an inhibitor or a substrate for P-gp (P-glycoprotein), BCRP (breast cancer resistant protein), and OCT2 (organic cation transporter 2) at 10 μmol/L. In vivo experimental data revealed that ABX-1431 inhibited MAGL with an ED₅₀ of 0.5±1.4 mg/kg (p.o.) and dose-dependently increased 2-AG levels in mouse brain. Pharmacokinetic studies in rats and dogs have indicated that ABX-1431 has a low to moderate systemic clearance, moderate volume of distribution, and high oral bioavailability (64% in rat, 57% in dog, Table 3). Additionally, pharmacodynamic effects of ABX-1431 were assessed by using a rat inflammatory pain model, demonstrating that ABX-1431 exhibited potent antinociceptive effects in a formalin paw test at a single oral dose of 3 mg/kg (a dose produced near complete MAGL inhibition and maximal elevation of 2-AG). Other pharmacological effects have not been reported yet in the literature. Positron emission tomography (PET) studies have shown that ABX-1431 inhibited MAGL in the brain in a dose-dependent manner. To the best of our knowledge, at least five different clinical trials have been tested for ABX-1431 (Table 4, www.clinicaltrials.gov). In phase 1 clinical studies, ABX-1431 was well-tolerated and safe, and the observed most common adverse effects were headache, somnolence, and fatigue. In a double-blind, randomized, placebo-controlled, cross-over phase 1b study, ABX-1431 showed positive effects on the symptoms of adult patients with Tourette’s syndrome. Currently, ABX-1431 is entering clinical phase 2 studies for the treatment of several neurological disorders such as neuromyelitis optica and multiple sclerosis (Table 4). The results have shown that ABX-1431 had positive effects for patients suffering from neurological diseases. Table 4 summarized current clinical studies of ABX-1431. Hopefully, ABX-1431 may have positive clinical results, to speed up the development of MAGL inhibitors.

| Study phase | Status | Study title | Condition or disease | Intervention/treatment |
|-------------|--------|-------------|----------------------|------------------------|
| Phase 1     | Active, not recruiting | A randomized, placebo-controlled, optimized titration study of ABX-1431 in adult patients with peripheral neuropathic pain. | Post herpetic neuralgia, Diabetic peripheral neuropathy, Small fibre neuropathy, Post-traumatic neuralgia | Drug: ABX-1431, Drug: placebo oral capsule |
| Phase 1     | Completed | A double-blind, placebo-controlled, crossover study to evaluate the safety and efficacy of ABX-1431 in patients with central pain. | Neuromyelitis optical spectrum disorder, Transverse myelitis, Multiple sclerosis, Longitudinally extensive transverse myelitis | Drug: ABX-1431 HCl, Drug: placebo |
| Phase 1     | Completed | An fMRI study in healthy volunteers to investigate the effects of ABX-1431 on experimental hyperalgesia and its neural correlates. | Pain | Drug: ABX-1431, Drug: placebo |
| Phase 1     | Terminated (recruitment challenges) | A single-dose study to evaluate the effects of ABX-1431 on gastric accommodation and nutrient volume tolerance in patients with functional dyspepsia. | Dyspepsia | Drug: ABX-1431, Drug: placebo |
| Phase 1     | Completed | A randomized, placebo-controlled, single-dose crossover study of ABX-1431 HCl in adult patients with tourette syndrome (TS) and chronic motor tic disorder. | Tourette syndrome, Chronic motor tic disorder | Drug: ABX-1431, Drug: placebo comparator |
| Phase 2     | Recruiting | A randomized, placebo-controlled study of ABX-1431 in adult patients with tourette syndrome or chronic motor tic disorder. | Tourette syndrome, Motor tic disorder | Drug: ABX-1431, Drug: placebo |
5.1.4. Ureas
Sanofi–Aventis reported a triazole urea compound SAR629 (22, Fig. 9), which acts as a potent covalent MAGL inhibitor. The X-ray data has demonstrated that the urea moiety of SAR629 interacted with the serine residue within the catalytic site of MAGL, followed by the formation of a carbamylated enzyme adduct and subsequent release of the triazole moiety (Fig. 10). SAR629 is proposed to irreversibly react with MAGL, however, the irreversibility still need to be examined. Subsequently, a series of urea-based MAGL inhibitors have been identified and optimized, such as compound ML30 (23, Fig. 9), showing high potency against hMAGL with an IC_{50} value of 0.54 nmol/L determined by the [\textsuperscript{3}H]-2-oleoyl glycerol hydrolysis assay. Further structural modifications of urea derivatives SAR629 and ML30 have been performed to improve the selectivity against MAGL, resulting in the identification of compounds JJKK-046 (24) and JJKK-048 (25, Fig. 9). Both 24 and 25 showed high inhibition activities against hMAGL with IC_{50} values of 0.56 and 0.36 nmol/L, respectively, which were determined by a natural substrate assay. Both compounds were selective over other serine hydrolases, including FAAH and ABHD. Of note, 25 was able to selectively increase 2-AG levels in rat brain without affecting AEA levels. Additionally, urea-based compounds 26 and 27 were reported to selectively inhibit MAGL at submicromolar concentrations (Fig. 9). As 26 and 27 were optimized from the scaffold of loratadine (28, a histamine H1 receptor antagonist, Fig. 9), they still maintained antagonistic activities against the histamine H1 receptor.

5.1.5. Arylthioamides
Arylthioamide derivatives such as CK16 (29) and CK37 (30) (Fig. 11) showed inhibition activities against MAGL with IC_{50} values of 355 and 154 nmol/L, respectively. Although the inhibition activities of these inhibitors are moderate, the low logP values make them favourable for the further development of MAGL inhibitors. In vivo experiments revealed that 29 slightly increased 2-AG levels, however, 30 dramatically elevated 2-AG levels. The discrepancy between those in vivo and in vitro experiments might be due to the stability of compounds 29 and 30. Rapid dilution studies have suggested that 30 might inhibit MAGL irreversibly via a covalent binding. To further investigate the inhibition mechanism, the authors conducted other studies such as the addition of dithiothreitol (DTT) studies and mutated hMAGL constructs studies, and found the potential formation of a DTT-

![Figure 9](image_url) Chemical structures of representative urea-based MAGL inhibitors 22–27 and loratadine 28 (histamine H1 receptor antagonist).

![Figure 10](image_url) (a) X-ray cocrystal structure of human MAGL (grey) with SAR629 (green), referred by PDB code 3JWE. (b) Key interactions of MAGL–SAR629, catalytic Ser132 covalently bound to SAR629. Hydrogen bonds are depicted as green dashed lines, whereas π−σ and σ−σ interactions are depicted as purple dashed lines.
sensitive covalent bond between compound 30 and Cys208 or Cys242 that are noncatalytic residues in MAGL88. According to their results, the formation of an adduct between inhibitor and the catalytic serine (Ser122) cannot be excluded.

5.2. Reversible inhibitors

5.2.1. Tetrahydrolipstatin (THL)-based inhibitors
THL (Fig. 12a) is an approved drug for the treatment of obesity by the inhibition of lipases. In 2003, THL was found to show high potency against DAGLs, enzymes that responsible for 2-AG biosynthesis (Fig. 1a)102. Subsequently, a series of THL-based analogues were synthesized and screened leading to the identification of a MAGL inhibitor OMDM169 (31) (Fig. 12a)89,103. OMDM169 inhibited MAGL with an IC50 of 0.89 μmol/L, and was more selective compared to its activity on DAGL (7-fold) and FAAH (>7-fold)89. Of note, the inhibition activity of OMDM169 against MAGL varies among different enzyme species. For example, OMDM16939 exhibited better activity against rat MAGL than mouse MAGL in the brain (Table 2). In addition, OMDM169 was shown to modestly increase 2-AG levels without affecting AEA levels in neuroblastoma cells and in paws of formalin-treated mice. Initially, OMDM169 was reported as an irreversible inhibitor against β-lactone, however, subsequent studies have implicated that OMDM169 might covalently and reversibly interact with MAGL due to the hydrolysis of enzyme-inhibitor adduct (Fig. 12b)104,105.

5.2.2. Isothiazolines
Isothiazoline derivatives are considered as promising scaffolds for MAGL inhibitors. Octhilinone (32)14 (Fig. 13a) was described to inhibit rat recombinant MAGL with an IC50 of 88 nmol/L through a partially reversible mechanism where enzyme recovery was observed after dilution. Subsequent structure modifications generated 33 and 34 by introduction of a N-substituted long hydrophobic alkyl group (33) or replacement of isothiazolineone moiety with benzothiazolinone (34, Fig. 13a)34. The inhibition potencies of 33 (IC50 = 43 nmol/L) and 34 (IC50 = 20 nmol/L) against MAGL were slightly improved in comparison with 32. The interaction mechanism of 32 was further investigated using a reducing agent DTT. The enzyme inhibition induced by 32 was blocked by the addition of DTT, but not other MAGL inhibitors14. The results indicated that 32 might form a reducible bond with amino acids in the active site of MAGL, which is different from the formation of a Michael addition product34. Accordingly, 32 was proposed to form a disulphide bond with MAGL (Fig. 13b).

5.2.3. Natural terpenoids
The naturally occurring terpenoids pristimerin (35) and euphol (36) are described as reversible MAGL inhibitors (Fig. 14)90. Compound 35 was reported to inhibit purified recombinant rat MAGL with an IC50 of 93 nmol/L and increase 2-AG but not AEA levels in the brain90. On the other hand, compound 36 (IC50 = 315 nmol/L, purified recombinant rat MAGL), less potent than 35, whilst it did not alter 2-AG concentrations in the brain90. According to the chemical structure, the quinone methide group (35) is capable of reacting with the cysteine to form a covalent intermediate106. To verify the inhibition mechanism of 35, rapid dilution assays were performed. The catalytic activity of MAGL was recovered after rapid dilution of the MAGL – 35 mixture, implicating a reversible inhibition mechanism. Moreover, time-course experiments and kinetic studies demonstrated that 35 inhibited MAGL in a rapid, reversible and non-competitive manner106. Terpenoids 35 and 36 were reported to occupy a common hydrophobic pocket located within the lid domain of MAGL and interact with the adjacent cysteines reversibly106. Notably, the discovery of terpenoids was important for the development of novel, potent and reversible MAGL inhibitors.

5.2.4. Amide-based derivatives
In 2010, Janssen Pharmaceutica107,108 patented a series of potent and reversible amide-based MAGL inhibitors. These inhibitors all inhibit rat recombinant MAGL with an IC50 of 88 nmol/L through a partially reversible mechanism where enzyme recovery was observed after dilution. Subsequent structure modifications generated 33 and 34 by introduction of a N-substituted long hydrophobic alkyl group (33) or replacement of isothiazolineone moiety with benzothiazolinone (34, Fig. 13a)34. The inhibition potencies of 33 (IC50 = 43 nmol/L) and 34 (IC50 = 20 nmol/L) against MAGL were slightly improved in comparison with 32. The interaction mechanism of 32 was further investigated using a reducing agent DTT. The enzyme inhibition induced by 32 was blocked by the addition of DTT, but not other MAGL inhibitors14. The results indicated that 32 might form a reducible bond with amino acids in the active site of MAGL, which is different from the formation of a Michael addition product34. Accordingly, 32 was proposed to form a disulphide bond with MAGL (Fig. 13b).

Figure 11 Chemical structures of representative arylthioamides-based MAGL inhibitors 29 and 30.

Figure 12 (a) Chemical structures of tetrahydrolipstatin (THL, olistat) and MAGL inhibitor 31 (OMDM169); (b) plausible action mechanism of OMDM169 on MAGL by the formation of an acyl enzyme intermediate.
possess piperazine and azetidine cycles with carbonyl groups in their structures (Fig. 15). The binding mode of these compounds was resolved by the X-ray crystallography using human MAGL and a representative compound 37. Structure optimization of 37 led to the generation of a high potent inhibitor 38 (IC$_{50}$ < 5 nmol/L), which was able to increase 2-AG levels in the rat brain homogenate. In 2013, compounds with a piperidine rather than piperazine ring were reported as MAGL inhibitors (e.g., compound 39 and 40; Fig. 15) in an US patent. A surrogate assay using 4-methylumbelliferyl butyrate as substrate was applied to evaluate the activity of these inhibitors on MAGL. The results revealed that these amide-based derivatives maintained high potency against MAGL with IC$_{50}$ lower than 5 nmol/L. To evaluate the in vivo efficacy, compound 39 (30 mg/kg, p.o.) was administrated to the complete Freund’s adjuvant (CFA)-induced cutaneous inflammation animal model, and ~20% reversal of hypersensitivity was observed after 5 h. Several other neuropathic pain animal models were also discussed in the patent, but no relative results were reported.

5.2.5. Other reversible inhibitors

In 2014, Hernande-Torres et al. found that compound c21 (41) was a potent, selective and reversible MAGL inhibitor with an IC$_{50}$ of 180 nmol/L against mouse MAGL (Fig. 16a). To evaluate in vivo efficacy of 41, the experimental allergic encephalomyelitis (EAE) mouse model was applied. EAE model is broadly studied as an animal model of human CNS demyelinating diseases, including multiple sclerosis and acute disseminated encephalomyelitis. Compound 41 alleviated the clinical progression of a multiple sclerosis mouse model without inducing undesirable CB$_1$-mediated side effects in disparate in vivo mouse models. More importantly, as a reversible inhibitor, 41 did not induce catalepsy or other motor impairments that had been observed by irreversible inhibitors. In 2014, Tuccinardi et al. identified benzoylpiperidine derivatives as a new type of reversible MAGL inhibitors by a virtual screening study. As a starting point, 42 (CL6a, Fig. 16a) proved to be a promising inhibitor with an IC$_{50}$ of 11.7 nmol/L against recombinant human MAGL. Probable binding poses from molecular modelling were used to guide the modification of 42, leading to the generation of compound 43 with improved potency on MAGL (IC$_{50}$ = 840 nmol/L, hMAGL; Fig. 16a). Meanwhile, compound 43 displayed a high MAGL selectivity over FAAH as well as antiproliferative activity in a few cancer cells. Further optimization of 43 by replacing the p-chlorophenyl ring with a biphenyl ring led to ~2-fold increase in its inhibition activity. This indicated that modification of the p-chlorophenyl region of these compounds is a promising strategy to improve the activity of inhibitors. Inspired by this, further structural optimization of 43 has been conducted by the same group, and identified compound 44 (Fig. 16a) as a new reversible MAGL inhibitor with high potency (IC$_{50}$ = 80 nmol/L) and selectivity.
IC50 values of MAGL against CB1R, CB2R, FAAH, ABHD6 and ABHD12 are all >10 μmol/L.93 Furthermore, the antiproliferative activities of 44 against aggressive cancer cells (e.g., human breast MDA-MB-231, colorectal HCT116, and ovarian CAOV3, OVCAR3 and SKOV3) have been evaluated, and 44 showed micromolar activities in these cells93. In addition, administration of 44 to mice (50 mg/kg, i.p.) significantly increased 2-AG levels in mouse brain and plasma, which confirmed the in vivo inhibitory activity of 4493. The preliminary results have indicated 44 was one of the most active and selective reversible MAGL inhibitors that has been reported in literature so far, however, the in vivo efficacy and ADME properties of this compound are still required to be explored and optimized. Recently, Aghazadeh Tabrizi et al.112 reported a diphenylpyrazole derivative 45, which has been characterized as a reversible mechanism-based MAGL inhibitor with good potency (Fig. 16a). 45 inhibited the activity of MAGL with an IC50 of 510 nmol/L, and exhibited a promising cell growth inhibitory activity in an antiproliferative assay in cancer cells that overexpress MAGL (e.g., OVCAR-3 and CAOV3). Moreover, the in vivo experiments have confirmed that 45 was effective for the treatment of neuropathic pain. In 2018, Takeda Pharmaceutical70 reported a series of novel piperazinyl pyrrolidine-2-one derivatives as reversible MAGL inhibitors using a structure-based drug design (SBDD) approach. Before the start of optimization, they identified a pyrrolidinone derivative 46 (Fig. 16b) through high-throughput screening campaign of their own compound library. However, compound 46 only showed moderate MAGL inhibitory activity (~ 10% inhibition at 10 μmol/L). To improve the inhibitory activity, the cocrystal structure of amide-based inhibitor 37 and MAGL was applied to guide the structure optimization, and thus, the pyrimidinyl piperazine form 37 was introduced to the 4 position at the pyrrolidinone ring of 46, leading to the generation of compound 47 (Fig. 16b). Compound 47 displayed significantly increased MAGL inhibitory activity with an IC50 of 140 nmol/L. Subsequently, a systematic SAR study on 47 was performed, and compound 48 (Fig. 16b) was identified as the most potent MAGL inhibitor with a subnanomolar inhibition activity (IC50 = 0.64 nmol/L)70. However, the crystallography study has shown that a cocrystal structure was observed only for (R)-48, whereas the cocrystal experiments were performed using racemic 48. This indicates that MAGL might recognize the chirality at position 4 of the pyrrolidinone ring. Although 48 exhibited high potency against MAGL, the metabolic stability in human liver microsomes was poor (181 μL/min/mg). To develop an orally available MAGL inhibitor, further optimization yielded (R)-49 (Fig. 16b), which had a good balance between inhibition activity (IC50 = 5.0 nmol/L) and metabolic stability (65 μL/min/mg)70. For selectivity assessment, (R)-49 is selective against MAGL over FAAH (IC50 > 10 μmol/L), however, the selectivity profiles over other enzymes like ABHD6 and ABHD12, or even on a proteome-wide selectivity profile are still required for (R)-49. PK studies have revealed that high exposure level of (R)-49 was observed in both plasma and the brain after 1 h of oral administration to mice. To evaluate the pharmacodynamics properties of (R)-49, 2-AG and AA concentrations were measured from mice brains after 1 h of oral administration. It has shown that (R)-49 dramatically reduced
AA (25%) and increased 2-AG (340%) in mice brain in vivo. Taken together, these compounds would provide new perspectives for the development of reversible inhibitors against MAGL.

6. Conclusion and perspectives

MAGL activity has central functions in various biological systems, particularly endocannabinoid signalling and AA metabolism. A number of studies in disease models, including intestinal inflammation, hepatic injury, insulin resistance, depression and stress, have indicated that inhibition of MAGL might serve as a powerful anti-inflammatory pharmacological strategy. Generally, two effects have been observed by MAGL inhibition: i) inducing anti-inflammatory and analgesic effects via activation of CB receptors by increasing 2-AG levels, and ii) reducing the production of prostaglandin levels by the lowered pool of AA. Based on these effects, development of MAGL inhibitors may have significant effects for inflammation and pain treatment.

Over the past decades, a number of MAGL inhibitors, particularly irreversible inhibitors, have been reported by both academia and pharmaceutical companies. Various strategies, including high-throughput screening, de novo design as well as optimization of existing compounds, have been applied to search for novel potent and selective MAGL inhibitors. The reported MAGL inhibitors were found to have a large number of therapeutic applications, including pain and inflammation, metabolic disorders, neurodegenerative pathologies, anxiety, epilepsy, and cancer. During the development of MAGL inhibitors, another important concern is the selectivity profile. Several other serine hydrolases such as FAAH, ABHD6 and ABHD12 have similar binding site properties with MAGL, whereas these enzymes exert different functions and have different endogenous substrates in human. Thus, to exclude the effects caused by inhibition of other enzymes and to highlight the key role of MAGL inhibition in the in vivo results, it is necessary to determine the selectivity profiles of reported MAGL inhibitors. Assisted by the powerful technique ABPP, several high potent and selective inhibitors (e.g., JZL184, KLM29 and MJN110) were developed and considered as useful tools to explore a wide range of positive effects that induced by MAGL inhibition in animal models. ABPP is an efficient chemical biology strategy that can be used to discover and optimize inhibitors for multiple enzymes both in vitro and in vivo. Importantly, ABPP provides a global map of interactions for covalent irreversible inhibitors, which is not limited to protein class or functions. Most of the reported MAGL inhibitors contain similar structural motifs. For example, piperidine or piperazine rings and urea or carbamate groups are often connected together (e.g., JZL184, MJN110, and SAR629). This common scaffold from MAGL inhibitors may interact with residues that involved in the catalytic process of MAGL. These residues might be the same portion that interact with the glycerol part of 2-AG, and are located in a very polar area of MAGL. In general, the known inhibitors can be classified into irreversible and reversible. Repeat administration of irreversible MAGL inhibitors to mice was reported to induce cross-tolerance with CB1 agonists. Accordingly, the utility of reversible inhibitors that temporarily block the enzyme would provide an important strategy for MAGL inhibitor development. However, pharmacological studies are still required to confirm that reversible inhibition of MAGL has better therapeutic effects and fewer side effects than irreversible inhibition. Of note, covalent binding modes do not necessarily result in irreversible inhibition (e.g., OMDM169), and hence, it is important to determine the exact kinetic properties of the inhibitors. Finally, Abide Therapeutics reported a first-in-class MAGL irreversible inhibitor ABX-1431, which is discovered and optimized by applying ABPP. To the best of our knowledge, this molecule has completed a placebo-controlled phase 1 study successfully, and is being evaluated in phase 2 clinical trials, which also showed promising preliminary results in patients with a neurological disease. It will be interesting to see whether chronic dose of ABX-1431 could induce functional antagonism of CB1Rs, or whether this molecule could mimic some psychoactive effects of CB1 agonists. Hopefully, the positive clinical results of ABX-1431 would ultimately provide a new alternative for patients with neurological disease such as Tourette’s syndrome, and thus, speed up the research of MAGL inhibitor development. To conclude, the therapeutic potential of MAGL inhibition is promising, but further research still requires.

Although MAGL inhibition was disclosed to have the potential for cancer treatment, how MAGL affects the metabolism of cancer cells is not well understood, and studies about the role of MAGL in cancer are also contradictory. Therefore, further comprehensive and systematic investigation on the role of MAGL in cancer is still required. Besides, according to the preclinical studies, MAGL inhibitors may have some side effects such as inducing CB1R desensitization in a chronic use. To avoid these potential adverse effects, lowering the dose of currently available irreversible inhibitors to ensure that MAGL is not completely inhibited will be required. Also, developing potent and selective MAGL reversible inhibitors would be another rational strategy. FAAH inhibitor BIA 10-2474 represents an encouraging lesson for the development of reversible MAGL inhibitors. BIA 10-2474, a covalent and irreversible inhibitor, has shown high potency and selectivity against FAAH in both in vitro and in vivo preclinical studies, however, it eventually led to failure in clinical trials. It has been postulated that off-target activities of BIA 10-2474 might have played a role. By application of the ABPP technique, Exbroeck et al. found that BIA 10-2474 displayed greater cross-reactivities with human serine hydrolases than other clinically tested FAAH inhibitor (e.g., PF04457845), suggesting the potential possibility to cause metabolic dysregulation in the nervous system. Therefore, the selectivity profile of MAGL inhibitors should also be seriously taken into account in the research field of MAGL inhibitor development in the future. A comprehensive selectivity profile is highly suggested for a MAGL inhibitor that is subject to clinical trials. During the development of MAGL inhibitors, their selectivity over FAAH is of particular importance, because inhibition of the off-target FAAH will induce the negative effects of dual activation of 2-AG and AEA metabolic pathways in vivo. In addition, the selectivity over ABHD6 and ABHD12 and other serine hydrolases linked in 2-AG degradation is also essential in the search of novel MAGL inhibitors. Although both ABHD6 and ABHD12 account only a small percentage of 2-AG hydrolysis, these enzymes are not well characterized yet, therefore, complete inhibition consequences are still difficult to be predicted. Currently, although several different reversible MAGL inhibitors were reported, their chemical structures are not versatile. Most of them contain similar motifs, particularly, piperidine or piperazine rings are often linked to amide groups, whereas aromatic or heteroaromatic groups of various sized are presented on the other sides of molecules. Therefore, to develop potent and selective reversible inhibitors, rational design from this general scaffold will be a good starting point. Additionally, several cocrystal structures of MAGL and its inhibitors have been reported, which would be beneficial for
researchers to rationally develop novel reversible inhibitors with better activity and selectivity. On the other hand, a high-throughput screening of a large variety of compound library will be another attractive approach to identify novel starting points for MAGL inhibitor development, as this approach has the potential to discover new scaffolds. Hopefully, a new class of reversible MAGL inhibitors with high potency and selectivity will be generated and could be developed as effective therapeutic agents in the future.

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Author contributions

Hui Deng and Weimin Li designed and wrote the paper.

Conflicts of interest

The authors have no conflicts of interest to declare.

References

1. Senior JR, Isselbacher KJ. Demonstration of an intestinal monoglyceride lipase: an enzyme with a possible role in the intracellular completion of fat digestion. J Clin Invest 1963;42:187–95.
2. Vaughan M, Berger JE, Steinberg D. Hormone-sensitive lipase and monoglyceride lipase activities in adipose tissue. J Biol Chem 1964;239:401–9.
3. Kupieczi FP. Partial purification of monoglyceride lipase from adipose tissue. J Lipid Res 1966;7:230–5.
4. Karlsson M, Contreras JA, Hellman U, Tornqvist H, Holm C. cDNA cloning, tissue distribution, and identification of the catalytic triad of monoglyceride lipase. Evolutionary relationship to esterases, lysophospholipases, and haloperoxidases. J Biol Chem 1997;272:27218–23.
5. 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;99:10819–24.
6. Maccarrone M, Bah I, Biró T, Cabral GA, Dey SK, Di Marzo V, et al. Endocannabinoid signaling at the periphery: 50 years after THC. Trends Pharmacol Sci 2015;36:277–96.
7. Baggelaar MP, Maccarrone M, van der Stelt M. 2-Arachidonoylglycerol: a signaling lipid with manifold actions in the brain. Prog Lipid Res 2018;71:1–17.
8. Nomura DK, Morrison BE, Blankman JL, Long JZ, Kinsey SG, Marcordes MC, et al. Endocannabinoid hydrolysis generates brain prostaglandins that promote neuroinflammation. Science 2013;343:809–13.
9. 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;5:37–44.
10. Chanda PK, Gao Y, Mark L, Bresh J, Strassle BW, Lu P, et al. Monoacylglycerol lipase activity is a critical modulator of the tone and integrity of the endocannabinoid system. Mol Pharmacol 2010;78:996–1003.
11. 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 2010;13:1113–9.
12. Glass M, Abool ME, Scotter EL. The endocannabinoid system as a target for the treatment of neurodegenerative disease. Br J Pharmacol 2010;160:480–98.
13. Bridges D, Ahmad K, Rice AS. The synthetic cannabinoid WIN55,212-2 attenuates hyperalgesia and allodynia in a rat model of neuropathic pain. Br J Pharmacol 2001;133:586–94.
14. Ibrahim MM, Deng H, Zvonok A, Cockayne DA, Kwan J, Mata HP, et al. Activation of CB2 cannabinoid receptors by AM1241 induces experimental neuropathic pain: pain inhibition by receptors not present in the CNS. Proc Natl Acad Sci U S A 2003;100:10529–33.
15. Kinsey SG, Nomura DK, O’Neal ST, Long JZ, Mahadevan A, Cravatt BF, et al. Inhibition of monoacylglycerol lipase attenuates nonsteroidal anti-inflammatory drug-induced gastric hemorrhages in mice. J Pharmacol Exp Ther 2011;338:795–802.
16. Woodhams SG, Wong A, Barrett DA, Bennett AJ, Chapman V, Alexander SP. Spinal administration of the monoacylglycerol lipase inhibitor IZL184 produces robust inhibitory effects on nociceptive processing and the development of central sensitization in the rat. Br J Pharmacol 2012;167:1609–19.
17. Douglass JD, Zhou YX, Wu A, Zdrojega JA, Gajda AM, Lackey AL, et al. Global deletion of MGL in mice delays lipid absorption and alters energy homeostasis and diet-induced obesity. J Lipid Res 2015;56:1153–71.
18. Nomura DK, Long JZ, Niessen S, Hoover HS, Ng SW, Cravatt BF. Monoacylglycerol lipase regulates a fatty acid network that promotes cancer pathogenesis. Cell 2010;140:89–91.
19. Vandevenoorde S, Saha B, Mahadevan A, Razdan RK, Pertwee RG, Martin BR, et al. Influence of the degree of unsaturation of the acyl side chain upon the interaction of analogues of 1-arachidonoylglycerol with monoacylglycerol lipase and fatty acid amide hydrolase. Biochem Biophys Res Commun 2005;337:104–9.
20. Rajesh M, Pan H, Mukhopadhyay P, Ratkai S, Osei-Hyiaman D, Haskó G, et al. Cannabinoid-2 receptor agonist HU-308 protects against hepatic ischemia/reperfusion injury by attenuating oxidative stress, inflammatory response, and apoptosis. J Leukoc Biol 2007;82:1382–9.
21. Calignano A, La Rana G, Giuffrida A, Piomelli D. Control of pain initiation by endogenous cannabinoids. Nature 1998;394:277–81.
22. Sánchez AJ, García-Merino A. Neuroprotective agents: cannabinoids. Clin Immunol 2012;142:57–67.
23. Di Marzo V, Goparaju SK, Wang L, Liu J, Bátkai S, Járai Z, et al. ABHD6 regulates endocannabinoid metabolism and integrity of the endocannabinoid system. Proc Natl Acad Sci U S A 2012;109:2611–6.
24. Kukkonen J, Hukkanen M, Eronen M, Conboy MI, Laari T, Pentikainen M, et al. Activation of CB2 cannabinoid receptors by AM1241 inhibits inflammation by ABHD6. Proc Natl Acad Sci U S A 2012;109:2611–6.
25. Di Marzo V, Goparaju SK, Wang L, Liu J, Bátkai S, Járai Z, et al. ABHD6 regulates endocannabinoid metabolism and integrity of the endocannabinoid system. Proc Natl Acad Sci U S A 2012;109:2611–6.
26. Alhouayek M, Masquelier J, Cani PD, Lambert DM, Muccioli GG. The role of monoacylglycerol lipase in experimental neuropathic pain: pain inhibition by receptors not present in the CNS. Proc Natl Acad Sci U S A 2003;100:10529–33.
27. Long JZ, Nomura DK, Cravatt BF. Characterization of monoacylglycerol lipase inhibition reveals differences in central and peripheral endocannabinoid metabolism. Chem Biol 2009;16:744–53.
28. Mills GB, Moolenaar WH. The emerging role of lysophosphatidic acid D2-glycerol ester in the control of macrophage activation and inflammation by ABHD6. Proc Natl Acad Sci U S A 2013;110:17558–63.
29. Alhouayek M, Masquelier J, Cani PD, Lambert DM, Muccioli GG. The role of monoacylglycerol lipase in experimental neuropathic pain: pain inhibition by receptors not present in the CNS. Proc Natl Acad Sci U S A 2003;100:10529–33.
30. Barabás J, Vinczevics P, Böde K, Ferrer JL, Wouters J, Lambert DM. Crystal structure of the human monoacylglycerol lipase, a key actor in endocannabinoid signaling. ChemBioChem 2010;11:218–27.
31. Schalk-Hihi C, Schubert C, Alexander R, Bayoumy S, Clemente JC, Deckman I, et al. Crystal structure of a soluble form of human monoglyceride lipase in complex with an inhibitor at 1.35 Å resolution. Protein Sci 2011;20:670–83.
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31. Granchi C, Caligiuri I, Minutolo F, Rizzolio F, Tuccinardi T. A patent review of monoacylglycerol lipase (MAGL) inhibitors (2013–2017). Expert Opin Ther Pat 2017;27:1341–51.

32. Viso A, Cisneros JA, Ortega-Gutiérrez S. The medicinal chemistry of agents targeting monoacylglycerol lipase. Curr Top Med Chem 2008;8:231–46.

33. Tornero H, Belfrage P. Purification and some properties of a monoacylglycerol-lipase-hydrolyzing enzyme of rat adipose tissue. J Biol Chem 1976;251:813–9.

34. King AR, 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;157:974–83.

35. Ghaoufi N, Tiger G, Razdan RK, Mahadevan A, Pertwee RG, Martin BR, et al. Inhibition of monoacylglycerol lipase and fatty acid amide hydrolase by analogues of 2-arachidonoylglycerol. Br J Pharmacol 2004;143:774–84.

36. Bisogno T, Melck D, De Petrocellis L, Di Marco V. Phosphatidic acid as the biosynthetic precursor of the endocannabinoid 2-arachidonoylglycerol in intact mouse microblasts cells stimulated with ionomycin. J Neurochem 1999;72:2113–9.

37. Blankman JL, Simon GM, Cravatt BF. A comprehensive profile of brain enzymes that hydrolyze the endocannabinoid 2-arachidonoylglycerol. Chem Biol 2007;14:1347–56.

38. Dinh TP, Kathuria S, Piomelli D. RNA interference suggests a primary role for monoacylglycerol lipase in the degradation of the endocannabinoid 2-arachidonoylglycerol. Mol Pharmacol 2004;66:1260–4.

39. Goparaju SK, Ueda N, Yamaguchi H, Yamamoto S. Anandamide amidohydrolase reacting with 2-arachidonoylglycerol, another cannabinoid receptor ligand. FEBS Lett 1998;422:69–73.

40. Savinainen JR, Kansanen E, Pantzar T, Nava-Paldanius D, Parkkari T, Lehtonen M, et al. Robust hydrolysis of prostaglandin e2-glycerol esters by human monoacylglycerol lipase (MAGL). Mol Pharmacol 2014;86:522–35.

41. Heier C, Taschler U, Radulovic M, Aschauer P, Eichmann TO, Grond S, et al. Monoacylglycerol lipases act as evolutionarily conserved regulators of non-oxidative ethanol metabolism. J Biol Chem 2016;291:11865–75.

42. Gulyas AI, Cravatt BF, Bracey MH, Dinh TP, Piomelli D, Boscia F, et al. Segregation of two endocannabinoid-hydrolyzing enzymes into pre- and postynaptic compartments in the rat hippocampus, cerebellum and amygdala. Eur J Neurosci 2004;20:441–58.

43. Labar G, Wouters J, Lambert DM. A review on monoacylglycerol lipase: at the interface between fat and endocannabinoid signalling. Curr Med Chem 2010;17:2588–607.

44. Zhong P, Pan B, Gao XP, Blankman JL, Cravatt BF, Liu QG. Genetic deletion of monoacylglycerol lipase alters endocannabinoid-mediated retrograde synaptic depression in the cerebellum. J Physiol 2011;589:4847–55.

45. Grabner GF, Eichmann TO, Wagner B, Gao Y, Farzi A, Taschler U, et al. Deletion of monoglyceride lipase in astrocytes attenuates lipopolysaccharide-induced neuroinflammation. J Biol Chem 2016;291:913–23.

46. Marr WR, Blankman JL, Horne EA, Thomaeez 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;13:951–7.

47. Bonventre JV, Huang Z, Taheri MR, O’Leary E, Li E, Moskowitz MA, et al. Reduced fertility and postischaemic brain injury in mice deficient in cytosolic phospholipase A2. Nature 1997;390:622–5.

48. Mechoulam R, Parker LA. The endocannabinoid system and the brain. Annu Rev Psychol 2013;64:21–47.

49. Tuczotte C, Chouinard F, Lefebvre JS, Flaman N. Regulation of inflammation by cannabinoids, the endocannabinoids 2-arachidonoyl-glycerol and arachidonoyl-ethanolamide, and their metabolites. J Leukoc Biol 2015;97:1049–70.

50. Glass CK, Saijo K, Winner B, Marchetto MC, Gage FH. Mechanisms underlying inflammation in neurodegeneration. Cell 2010;140:918–34.

51. Klein TW. Cannabinoid-based drugs as anti-inflammatory therapeutics. Nat Rev Immunol 2005;5:400–11.

52. Ng SC, Chan FK. NSAID-induced gastrointestinal and cardiovascular injury. Curr Opin Gastroenterol 2010;26:611–7.

53. Cannon CP, Cannon PJ. Physiology: COX-2 inhibitors and cardiovascular risk. Science 2012;336:1386–7.

54. Piro JR, Benjamin DI, Duer MJ, Pi Y, Gonzales C, Wood KM, et al. A dysregulated endocannabinoid–eicosanoid network supports pathogenesis in a mouse model of Alzheimer’s disease. Cell Rep 2012;1:617–23.

55. Quarta C, Mazza R, Obici S, Pasquali R, Pagotto U. Energy balance regulation by endocannabinoids at central and peripheral levels. Trends Mol Med 2011;17:518–26.

56. Cota D, Marsicano G, Tschöp M, Grübner Y, Flachskamm C, Schubert M, et al. The endogenous cannabinoid system affects energy balance via central orexigenic drive and peripheral lipogenesis. J Clin Invest 2003;112:423–31.

57. Quarta C, Bellochio L, Mancini G, Mazza R, Cervino C, Braulke LI, et al. CB2 signaling in forebrain and sympathetic neurons is a key determinant of endocannabinoid actions on energy balance. Cell Metab 2010;11:273–85.

58. Christensen R, Kristensen PK, Bartels EM, Bliddal H, Astrup A. Efficacy and safety of the weight-loss drug rimonabant: a meta-analysis of randomised trials. Lancet 2007;370:1706–13.

59. Berdan CA, Erson KA, Burritt NE, Corkey BE, Deeney JT. Inhibition of monoacylglycerol lipase activity decreases glucose-stimulated insulin secretion in INS-1 (832/13) cells and rat islets. PLoS One 2016;11:e0149008.

60. 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;18:846–56.

61. Ye L, Zhang B, Sevirig EU, Tao KX, Liu XH, Ling Y, et al. Monoacylglycerol lipase (MAGL) knockdown inhibits tumor cells growth in colorectal cancer. Cancer Lett 2011;307:16–17.

62. Zhang J, Liu Z, Lian Z, Liao R, Chen Y, Qin Y, et al. Monoacylglycerol lipase: a novel potential therapeutic target and prognostic indicator for hepatocellular carcinoma. Sci Rep 2016;6:35784.

63. Zhu W, Zhao Y, Zhou J, Wang X, Pan Q, Zhang N, et al. Monoacylglycerol lipase promotes progression of hepatocellular carcinoma via NF-κB-mediated epithelial-mesenchymal transition. J Hematol Oncol 2016;9:127.

64. Sticht MA, Long JZ, Rock EM, Limebeer CL, Mechoulam R, Cravatt BF, et al. Inhibition of monoacylglycerol lipase protects rats from SARS-CoV-2 infection. J Biol Chem 2021;296:7059–70.

65. Franchi C, Rizzolio F, Bordoni V, Caligiuri I, Manera C, Macchia M, et al. 4-Arylidene-2-methyl-5H-oxazol-3-one as a new scaffold for selective reversible MAGL inhibitors. J Enzyme Inhib Med Chem 2016;31:137–46.

66. Butler CR, Beck EM, Harris A, Huang Z, McAllister LA, Am Ende CW, et al. Azetidine and piperidine carboxamides as efficient, covalent inhibitors of monoacylglycerol lipase. J Med Chem 2017;60:9860–73.

67. Labar G, Bauvois C, Muccioli GG, Wouters J, Lambert DM. Disulfiram is an inhibitor of human purified monoacylglycerol lipase, the enzyme regulating 2-arachidonoylglycerol signaling. Chem-BioChem 2007;8:1293–7.
arachidonoylglycerol impairs learning and memory performance while producing antinoceptive activity in rodents. Sci Rep 2015;5:7642.

69. King AR, Duranti A, Tontini A, Rivara S, Rosengarth A, Clapper JR, et al. URBD62 inhibits monoacylglycerol lipase and selectively blocks 2-arachidonoylglycerol degradation in intact brain slices. Chem Biol 2007;14:1357–65.

70. Aida J, Fushimi M, Kusumoto T, Sugiyama H, Arimura N, Ikeda S, et al. Design, synthesis, and evaluation of piperezinyl pyrrolidin-2-ones as a novel series of reversible monoacylglycerol lipase inhibitors. J Med Chem 2018;61:9205–17.

71. 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;53:2413–24.

72. Cravatt BF, Wright AT, Kozarich JW. Activity-based protein profiling: from enzyme chemistry to proteomic chemistry. Annu Rev Biochem 2008;77:383–414.

73. Niphakis MJ, Cravatt BF. Enzyme inhibitor discovery by activity-based protein profiling. Annu Rev Biochem 2014;83:341–77.

74. Liu Y, Patricelli MP, Cravatt BF. Activity-based protein profiling: the serine hydrolases. Proc Natl Acad Sci U S A 1999;96:14694–9.

75. Chang JW, Cognetta III AB, Niphakis MJ, Cravatt BF. Proteome-wide reactivity profiling identifies diverse carbamate chemotypes tuned for serine hydrolase inhibition. ACS Chem Biol 2013;8:1590–9.

76. Cisar JS, Weber OD, Clapper JR, Blankman JL, Henry CL, Chang JW, Cognetta III AB, Niphakis MJ, Cravatt BF. Activity-based protein profiling. Annu Rev Biochem 2014;83:341–77.

77. Sharma R. Enzyme inhibition and bioapplications. Rijeka, Croatia: InTech; 2012.

78. Ahn K, Johnson DS, Fitzgerald LR, Liimatta M, Arendse A, Mangieri R, et al. An endocannabinoid mechanism for stress-induced analgesia. Br J Pharmacol 2014;171:730–4.

79. Saario SM, Salo OM, Nevalainen T, Poso A, Laitinen JT, Jarvinen T, et al. Characterization of the sulfhydryl-sensitive site in the enzyme responsible for hydrolysis of 2-arachidonoylglycerol in rat cerebellar membranes. Chem Biol 2005;12:649–56.

80. Matuszak N, Muccioli GG, Labar G, Lambert DM. Synthesis and in vitro evaluation of N-substituted maleimide derivatives as selective monoglyceride lipase inhibitors. J Med Chem 2009;52:7410–20.

81. Kapanda CN, Muccioli GG, Labar G, Poupaert JH, Lambert DM. Bis[dialkylamino(heterocarbonyl)]disulfides as potent and selective monoglyceride lipase inhibitors. J Med Chem 2009;52:7310–4.

82. Hohmann AG, Suplita RL, Bolton NM, Neely MH, Figley D, Mangieri R, et al. An endocannabinoid mechanism for stress-induced analgesia. Nature 2005;435:1108–12.

83. Aaltenon N, Savinainen JR, Ribas CR, Rönkkö K, Kuusisto A, Korhonen J, et al. Piperazine and piperedine triazole ureas as ultra-potent and highly selective inhibitors of monoacylglycerol lipase. Chem Biol 2013;20:379–90.

84. Chang JW, Niphakis MJ, Lum KM, Cognetta III 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;19:579–88.

85. Niphakis MJ, Cognetta III 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;4:1322–32.

86. McAllister LA, Butler CR, Mente S, O’Neill SV, Fonseca KR, Piro JR, et al. Discovery of trifluoromethylyl glycol carboxamates as potent and selective covalent monoacylglycerol lipase (MAGL) inhibitors for treatment of neuroinflammation. J Med Chem 2018;61:3008–26.

87. Morera L, Labar G, Ortar G, Lambert DM. Development and characterization of endocannabinoid hydrolases FAAH and MAGL inhibitors bearing a benzo triazol-1-yl carboxamide scaffold. Bioorg Med Chem 2012;20:6260–75.

88. Kapanda CN, Masquelier J, Labar G, Muccioli GG, Poupaert JH, Lambert DM. Synthesis and pharmacological evaluation of 2,4-dinitroarylthiocarbamate derivatives as novel monoacylglycerol lipase inhibitors. J Med Chem 2012;55:5774–83.

89. Bisogno T, Ortar G, Petrovino S, Morera E, Palazzo E, Nalli M, et al. Discovery of a potent inhibitor of 2-arachidonoylglycerol hydrolysis with antinoceptive activity in vivo. Biochem Biophys Acta 2009;1791:53–60.

90. 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;16:1045–52.

91. Hernández-Torres G, Cipriano M, Hédén E, Björklund E, Canales A, Zian D, et al. A reversible and selective inhibitor of monoacylglycerol lipase ameliorates multiple sclerosis. Angew Chem Int Ed Engl 2014;53:13765–70.

92. Granchi C, Rizzolio F, Palazzolo S, Carmignani S, Macchia M, Saccamanni G, et al. Structural optimization of 4-chlorobenzoylpiperidine derivatives for the development of potent, reversible, and selective monoacylglycerol lipase (MAGL) inhibitors. J Med Chem 2016;59:10299–314.

93. Granchi C, Lapillo M, Glasmacher S, Bononi G, Licari C, Poli G, et al. Optimization of a benzoylpiperidine class identifies a highly potent and selective reversible monoacylglycerol lipase (MAGL) inhibitor. J Med Chem 2019;62:1932–58.

94. Vandevoorde S, Jonsson KO, Labar G, Persson E, Lambert DM, Fowler CJ. Lack of selectivity of URBD62 for 2-oleoylglycerol compared to anandamide hydrolysis in vitro. Br J Pharmacol 2007;150:186–91.

95. Kinsley SG, Wise LE, Ramesh D, Abdullah R, Selley DE, Cravatt BF, et al. Repeated low-dose administration of the monoacylglycerol lipase inhibitor JZL184 retains cannabionoid receptor type 1-mediated antinoceptive and gastroprotective effects. J Pharmacol Exp Ther 2013;345:492–501.

96. Zhang Z, Wang W, Zhong P, Liu SJ, Long JZ, Zhao L, et al. Blockade of 2-arachidonoylglycerol hydrolysis produces antidepressant-like effects and enhances adult hippocampal neurogenesis and synaptic plasticity. Hippocampus 2015;25:16–26.

97. 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.

98. Ignatowska-Jankowska BM, Ghosh S, Crowe MS, Kinsley SG, Niphakis MJ, Abdullah RA, et al. In vivo characterization of the highly selective monoacylglycerol lipase inhibitor KML29: antinoceptive activity without cannabimimetic side effects. Br J Pharmacol 2014;171:1392–407.

99. Jiang M, van der Stelt M. Activity-based protein profiling delivers selective drug candidate ABX-1431, a monoacylglycerol lipase inhibitor, to control lipid metabolism in neurological disorders. J Med Chem 2018;61:9059–61.

100. Bertrand T, Augé F, Houtmann J, Rak A, Valle F, Mikol V, et al. Structural basis for human monoglyceride lipase inhibition. J Mol Biol 2010;396:663–73.

101. Patel IZ, Ahenkorah S, Vaura M, Staszewski M, Adams Y, Laitinen T, et al. Loratadine analogues as MAGL inhibitors. Bioorg Med Chem Lett 2015;25:1436–42.

102. Bisogno T, Howell F, Williams G, Minassi A, Cascio MG, Ligresti A, et al. Cloning of the first sn-1-DAG lipases points to the spatial and temporal regulation of endocannabinoid signaling in the brain. J Cell Biol 2003;163:463–8.

103. Ortar G, Bisogno T, Ligresti A, Morera E, Nalli M, Di Marzo V. Tetrahydrocannabinol analogues as modulators of endocannabinoid 2-arachidonoylglycerol metabolism. J Med Chem 2008;51:6970–9.
104. Borgstrom B. Mode of action of tetrahydrolipstatin: a derivative of the naturally occurring lipase inhibitor lipstatin. *Biochim Biophys Acta* 1988;962:308–16.

105. Hadváry P, Sidler W, Meister W, Vetter W, Wolfer H. The lipase inhibitor tetrahydrolipstatin binds covalently to the putative active site serine of pancreatic lipase. *J Biol Chem* 1991;266:2021–7.

106. Bolton JL, Turnipseed SB, Thompson JA. Influence of quinone methide reactivity on the alkylation of thiol and amino groups in proteins: studies utilizing amino acid and peptide models. *Chem Biol Interact* 1997;107:185–200.

107. Janssen Pharmaceutica NV. Heteroaromatic and aromatic piperazinyl azetidinyl amides as monoacylglycerol lipase inhibitors. 2010 Oct 28. WO2010124122.

108. Janssen Pharmaceutica NV. Heteroaromatic and aromatic piperazinyl azetidinyl amides as monoacylglycerol lipase inhibitors. 2010 Oct 28. WO2010124121.

109. Janssen Pharmaceutica NV. Monoacylglycerol lipase inhibitors for the treatment of metabolic diseases and related disorders. 2013 Apr 04. WO2013049293.

110. Connolly PJ, Bian HY, Li X. Piperidin-4-yl-azetidine diamides as monoacylglycerol lipase inhibitors. 2012 Apr 26. WO2012054716A1.

111. Tuccinardi T, Granchi C, Rizzolio F, Caligiuri I, Battistello V, Toffoli G, et al. Identification and characterization of a new reversible MAGL inhibitor. *Bioorg Med Chem* 2014;22:3285–91.

112. Aghazadeh Tabrizi M, Baraldi PG, Baraldi S, Ruggiero E, De Stefano L, Rizzolio F, et al. Discovery of 1,5-diphenylpyrazole-3-carboxamide derivatives as potent, reversible, and selective monoacylglycerol lipase (MAGL) inhibitors. *J Med Chem* 2018;61:1340–54.

113. van Esbroeck AC, Janssen AP, Cognetta III AB, Ogasawara D, Shpak G, van der Kroeg M, et al. Activity-based protein profiling reveals off-target proteins of the FAAH inhibitor BIA 10-2474. *Science* 2017;356:1084–7.