Discovery of reversible monoacylglycerol lipase inhibitors

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Chapter 1

General Introduction

1.1 The endocannabinoid system

The endocannabinoid system (ECS), well known as the target of Δ^9-tetrahydrocannabinol (THC), the psychoactive component of marijuana, is a signaling network that modulates a diverse range of physiological processes including nociception, behavior, cognitive function, appetite, metabolism, motor control, memory formation, and inflammation.[1-3] There are two main endocannabinoids, 2-arachidonoylglycerol (2-AG) and *N*-arachidonoylethanolamine (anandamide), which act through membrane-bound G-coupled protein receptors (mainly CB_1 and CB_2) to alter these varied aspects of mammalian physiology.[4, 5] As shown in figure 1, the endogenous signaling lipid 2-AG is synthesized by diacylglycerol lipases (DAGLα and DAGLβ) which catalyze the *sn*-1-specific hydrolysis of diacylglycerol (DAG)[6, 7], while anandamide is synthesized by initial generation of *N*-arachidonoyl phosphatidylethanolamine followed by several postulated routes, such as *N*-acylphosphatidylethanolaminephospholipase D (NAPE-PLD) or α/β-hydrolase 4 (ABHD4)[8] mediated pathways. The main degradation enzyme for 2-AG is

monoacylglycerol lipase (MAGL), which hydrolyzes around 85% of 2-AG in the brain to give arachidonic acid (AA) and glycerol.[9] Together with ABHD6 and ABHD12, those enzymes response for 98% of the 2-AG hydrolysis. The key enzyme for the hydrolysis of anandamide is fatty acid amide hydrolase (FAAH).[10]

Initial drug development targeting the ECS led to several marketed drugs, such as Marinol® (Δ^9-tetrahydrocannabinol, THC), Epidiolex® (cannabidiol, CBD) and Cesamet® (Nabilone). These cannabinoid-based drugs directly activate cannabinoid receptors and, however, can also produce central side effects. Rimonabant®, a non-cannabinoid-based inverse agonist for CB_1 receptor which was marketed as anorectic anti-obesity drug, was withdrawn from the market due to psychological side effects.[11] Increasing the levels of endocannabinoids 2-AG or anandamide through inhibiting the metabolic enzymes MAGL or FAAH provides an alternative way to activate CB receptors. To date, several selective MAGL and FAAH inhibitors have entered clinical trials, however, none reached the market yet.[12-15]

1.2 Monoacylglycerol lipase (MAGL)

Monoacylglycerol lipase (MAGL or MGLL) is a serine hydrolase that catalyzes the hydrolysis of saturated or unsaturated monoacylglycerides to give free fatty acid and glycerol.[16, 17] It was initially discovered in the intestine and adipose tissue of rats.[18, 19] Later on, it was found to be the principal degradative enzyme for the endocannabinoid 2-AG in the brain.[20] The endogenous signaling lipid 2-AG is a full agonist for the cannabinoid receptors CB1 and CB2, which are the main receptors through which 2-AG exerts its physiological effects.[5, 21, 22] Besides this, 2-AG is also an important intermediate in lipid metabolism. Degradation of 2-AG leads to a major release of arachidonic acid (AA), which is the precursor of pro-inflammatory prostaglandins in the brain, liver and lung.[23] Therefore, MAGL is recognized as a critical player for regulating both the endocannabinoid and eicosanoid signaling pathways.

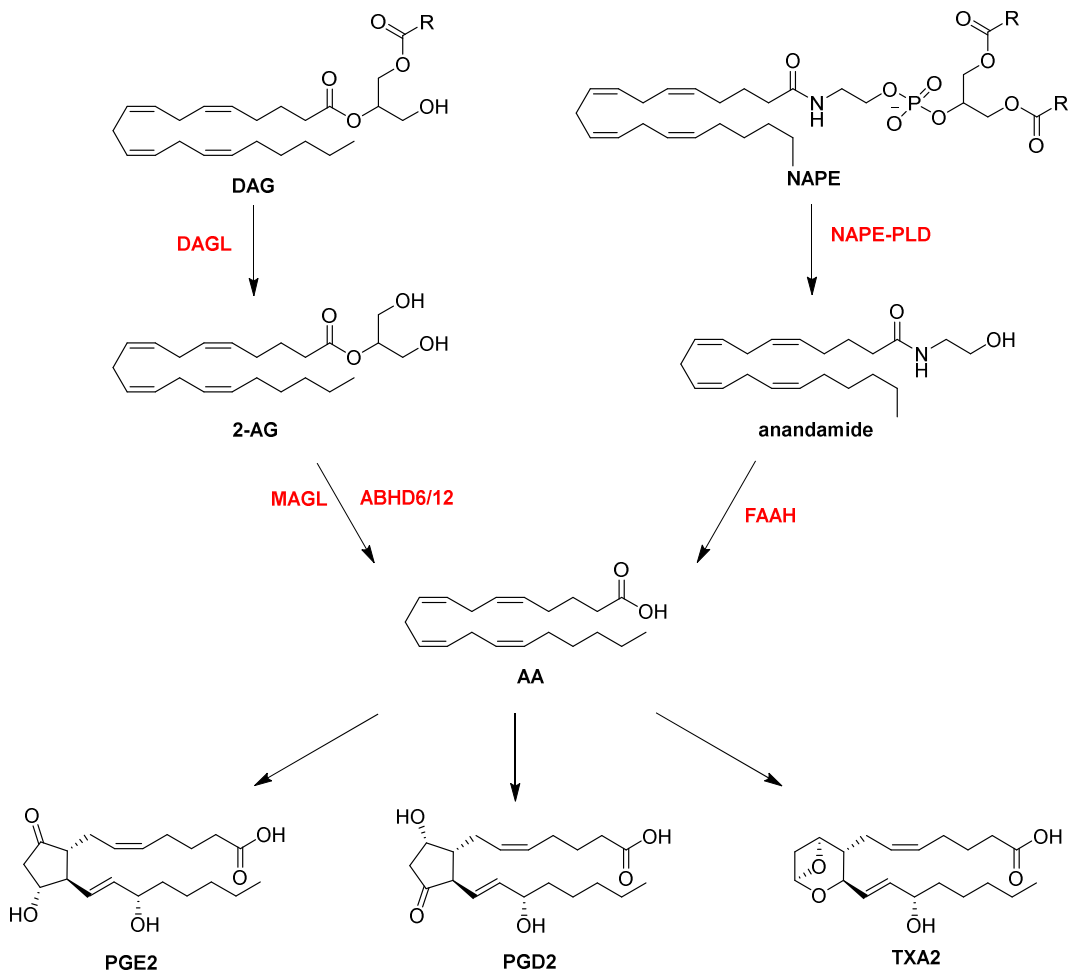

Figure 1. Biosynthesis and degradation of endocannabinoids 2-AG and anandamide. DAG: diacylglycerol; NAPE: N-acylphosphatidylethanolamine; 2-AG: 2-arachidonoylglycerol; AA: arachidonic acid; PGE2: prostaglandin E2; PGD2: prostaglandin D2; TXA2: thromboxane A2; DAGL: diacylglycerol lipases; NAPE-PLD: N-acylphosphatidylethanolaminephospholipase D; MAGL: monoacylglycerol lipase; ABHD6/12: α/β-hydrolase-domain containing protein 6/12; FAAH: fatty acid amide hydrolase.

MAGL is a membrane-associated enzyme which exists in two splicing forms with molecular weight of 33 and 35 kDa.[24] Several MAGL crystal structures have been reported and it has been described as a dimer.[14, 25-27] The catalytic triad of MAGL is formed by a Ser-Asp-His commonly found in the serine hydrolase family, which in the human ortholog is constituted by Ser122, Asp239 and His269.[28] The His activates Ser122, which functions as a nucleophile to attack the carbonyl of the substrate. Besides the catalytic triad, the substrate binding site comprises a large hydrophobic tunnel (ACB pocket) in which the acyl chain binds and cytoplasmic access channel (CA channel), which functions as the exit channel for the hydrophilic glyceryl moiety.[14]

The MAGL catalytic mechanism consists of two different phases: the hydrolysis of the substrate and the reactivation of the enzyme (Figure 2).[29] First, the substrate binds into the active site of MAGL and the carbonyl of the substrate is anchored to the proper position by forming two hydrogen bounds with MAGL (Michaelis-Menten Complex). Next, the carbonyl of the ester is attacked by the catalytic serine residue of MAGL, which results in a tetrahedral transition state with an anion. Next, the glycerol will be released and a covalent acyl-enzyme adduct (ester) is formed. Finally, the enzyme will be reactivated by hydrolysis of the ester by an activated water molecule. AA will be released and free MAGL.

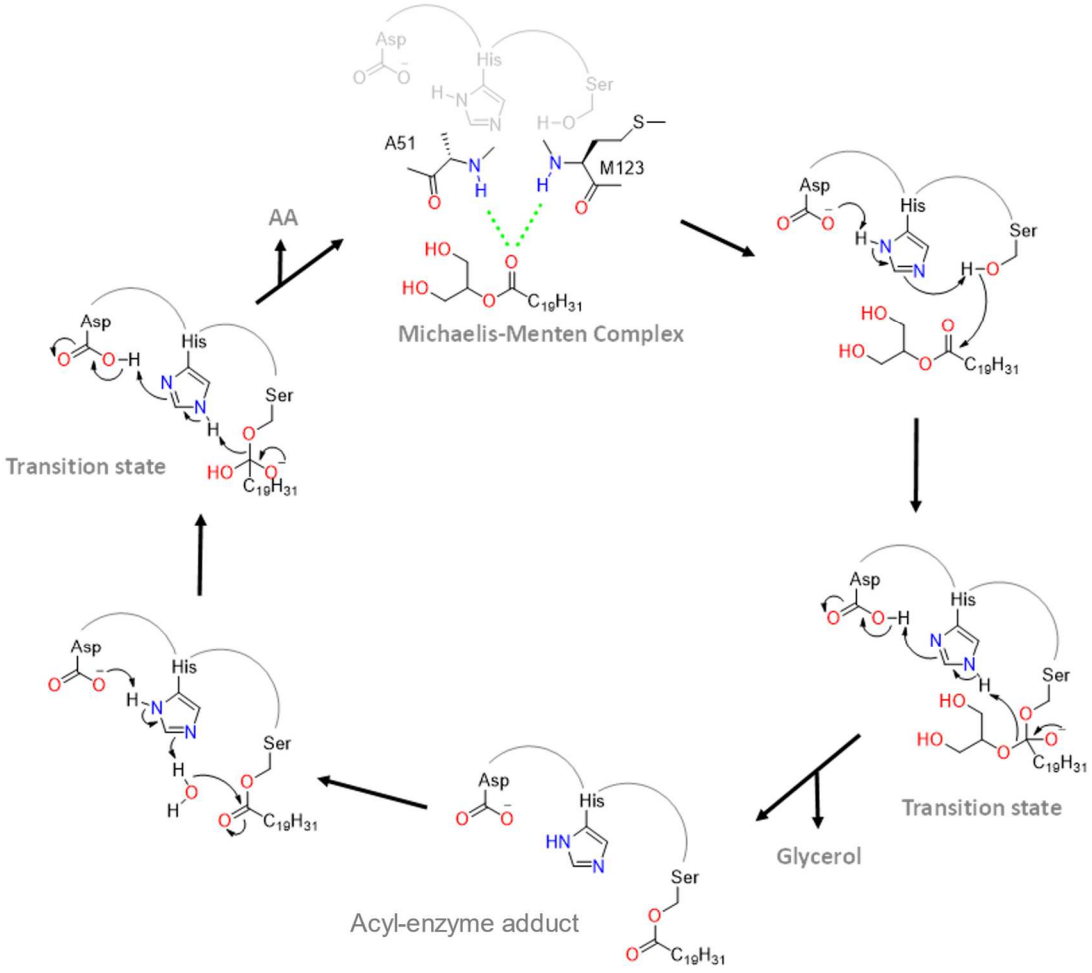

Figure 2. Catalytic mechanism of MAGL-mediated hydrolysis of 2-AG.

1.3 Therapeutic applications of MAGL inhibition

MAGL is considered as a promising drug target for a number of diseases due to its important roles in regulating both endocannabinoid and eicosanoid signaling pathways.

Acute MAGL inhibition with the selective inhibitor JZL184 (**1**, Figure 3) has been shown to exhibit a wide range of beneficial effects in various animal models of pain, inflammation, emesis, anxiety, opiate-induced withdrawal symptoms, colitis, neurodegeneration, inflammation-induced lung and liver injury, and cancer pathogenicity.[30-34]

1.3.1 Pain

Cannabinoids have analgesic effects due to their activation of the CB1 receptor. MAGL inhibition indirectly activates the CB1 receptor by increasing 2-AG levels. Not surprisingly, MAGL inhibition elicits also CB1-dependent antinociceptive effects in various mouse models of pain, including noxious chemical, inflammatory, thermal, and neuropathic pain.[35-37] Of note, MAGL inhibition by covalent irreversible inhibitors desensitized the CB1 receptor, which may lead to a loss of analgesia upon chronic administration.[38, 39] Using a lower dose of an irreversible inhibitor or a reversible inhibitor may prevent the induction of tolerance. Alternatively, administration of MAGL inhibitor JZL184 in combination with diclofenac (a cyclooxygenase inhibitor) has shown to reduce neuropathic pain with minimal adverse effects.[40]

1.3.2 Neuroinflammation and neurodegenerative diseases

During neuroinflammation, activated microglia and astrocytes produce proinflammatory cytokines and chemokines, and the blood−brain barrier is deteriorated. Persistent neuroinflammation can result in neuronal death and ultimately contribute to neurodegeneration.[41] MAGL inhibition has been shown to have beneficial effects in multiple neuroinflammatory processes. It was shown that MAGL hydrolysis of 2-AG provides the major pool of AA for the generation of neuroinflammatory eicosanoids in the brain. Pharmacological inhibition or genetic ablation of MAGL activity decreased lipopolysaccharide (LPS)-induced pro-inflammatory eicosanoid production, such as prostaglandin E_2 (PGE_2), PGD_2, PGF_2 and thromboxane B_2 (TXB_2), through CB receptor-independent mechanism.[31] Abolishment of MAGL activity also provided neuroprotective effects against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-

induced dopaminergic neurodegeneration, a model of Parkinson's disease. MAGL inhibition reduced dopamine loss and lowered pro-inflammatory eicosanoids and suppressing neuroinflammation.[42] In addition, inactivation of MAGL suppresses proinflammatory response and reduces production and accumulation of β-amyloid (Aβ) and improves cognitive function in the in a PS1/APP^{+} mouse model and 5XFAD mouse models of Alzheimer's disease.[33, 43]

1.3.3 Inflammatory tissue injury

Several studies have revealed that MAGL inhibition may have therapeutic effects in peripheral inflammatory tissue injury. MAGL inactivation lowered hepatic inflammation caused by ischemia-reperfusion (I/R) injury through reducing neutrophil infiltration, inflammatory cytokines, and reactive oxygen stress, and this hepatoprotective effect appeared to be due to a combination of enhanced CB2 signaling and lower eicosanoid levels. MAGL inhibition was also protective in the carbon tetrachloride and galactosamine/LPS models of liver injury in mice.[44] In addition, protective effects were also observed in LPS-induced acute lung injury model where MAGL inhibition reduced leukocyte migration into the lungs, vascular permeability, and inflammatory cytokine and chemokine levels in bronchoalveolar lavage fluid.[34]

1.4 Drug discovery of MAGL inhibitors

1.4.1 ABX-1431

Drug discovery for disorders of the central nervous system (CNS) is hard. Several factors contribute to the daunting task to discover novel therapies for brain diseases. First and foremost, there is a lack of validated therapeutic targets largely because of our limited understanding of the function of the brain in health and disease. Once a potential suitable target has been identified, the optimization of small molecules into drug candidates is complicated by the strict physicochemical properties required to pass the blood−brain barrier and to minimize efflux by membrane transporters. Furthermore, the determination of the target-interaction landscape (i.e., its selectivity profile) of the drug

in human brain is essential to avoid disasters as recently witnessed with fatal phase 1 clinical trial of BIA 10-2474. A volunteer died due to an overdose of BIA 10-2474. Thus, studies enabling target and off-target engagement in the brain are essential to guide drug discovery and development.[45-48]

Several academic groups and pharmaceutical companies have developed MAGL inhibitors that have a reversible or irreversible mode-of-action.[49-51] Irreversible inhibitors that covalently interact with the catalytic serine (Ser122) of MAGL, may achieve higher potency and sustained inactivation of the enzyme, thereby putting less demand on the pharmacokinetic properties. Determination of the selectivity profile of mechanism-based covalent inhibitors is, however, essential because other proteins from the same enzyme family of the primary target may also react with the warhead of the experimental drug in the same fashion. This could lead to unwanted side effects or toxicity. Thus, assessment of the interaction profile of the covalent inhibitor in human cells and brain is important.

ABX-1431 (**3**, Figure 3) is an irreversible MAGL inhibitor and was initially discovered by Abide Therapeutics which was acquired by Lundbeck B.V.. During the drug discovery process, activity-based protein profiling (ABPP) was utilized as the central technology for the discovery, optimization, and profiling of drug candidates. Competitive ABPP is an efficient chemical biology approach to study target engagement and interaction-landscape of covalent irreversible inhibitors in living systems.[47, 48] It makes use of broad-spectrum chemical probes that report on the abundance of active enzymes in lysates, (human) cells, or even intact animals. The interaction of a small molecule with endogenously expressed enzymes, including all post-translational modifications, protein−protein interactions in the presence of endogenous substrates, can be assessed in one single experiment. ABPP makes use of activity-based probes consisting of warhead, recognition element, and reporter group. A fluorescent reporter group is used for gel-based ABPP, whereas a biotin reporter allows mass spectrometry (MS)-based identification of the interacting proteins.

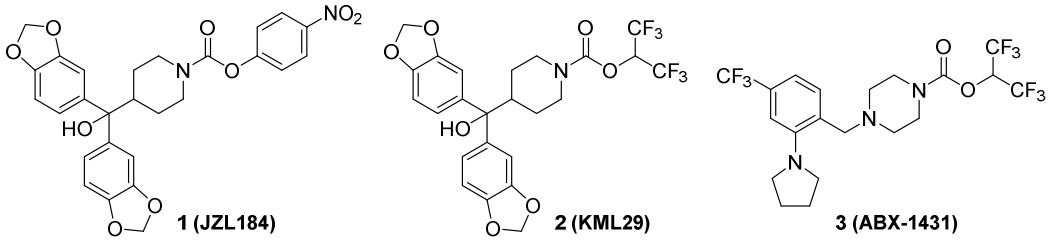

Figure 3. Chemical structure of MAGL inhibitors JZL184, KML29 and ABX-1431.

Cisar et al. used the prototypical fluorophosphonate (FP)-based probes to assess the interaction of their MAGL inhibitors on the serine hydrolase family.[13] JZL184 and KML29 (**1** and **2**, Figure 3) were used as a starting point for the rational design of novel MAGL inhibitors. Careful optimization of the activity and selectivity using gel-based ABPP with multiple human proteomes and rodent brain homogenates led to the discovery of ABX-1431, which was selected as the lead compound for clinical evaluation. ABX-1431 is a potent human MAGL inhibitor with an average IC_{50} of 14 nM that only cross-reacts to a minor extent with ABHD6, PLA2G7, and some carboxyl esterases. The compound maintained activity and selectivity in human cellular assays and in human prefrontal cortex proteomes was determined by MS-based ABPP. ABX-1431 is a lipophilic molecule and has a basic amine, yet it has only weak hERG channel activity with an IC_{20} of 7 μM. The compound did not display any significant activity against a panel of common off-targets and has low propensity to CYP-inhibition. ABX-1431 demonstrated acceptable pharmacokinetics in rodents and dogs. It inhibited MAGL activity with an ED_{50} of 0.5−1.4 mg/kg (po) and dose dependently increased brain 2-AG levels in mouse brain. A rat inflammatory pain model was used to assess the pharmacodynamics effect. ABX-1431 demonstrated potent antinociceptive effects in a formalin paw test at a dose that produced near complete MAGL inhibition and maximal elevation of 2-AG. Other pharmacological effects were not (yet) described. Currently, ABX-1431 is being tested in different clinical trials (www.clinicaltrials.gov). Notably, it has successfully completed phase 1 clinical trials. The compound was generally well-tolerated and safe. The most commonly observed adverse effects were headache, somnolence, and fatigue. It inhibited MAGL in the brain in a dose-dependent manner as demonstrated with a PET study. Importantly, in a randomized, double-blind,

placebo-controlled crossover, exploratory phase 1b study, ABX-1431 was able to show a positive impact on key measures of symptoms in adult patients with the syndrome of Gilles de la Tourette. It has now entered another phase 1b clinical trial for posttraumatic stress disorder (NCT04597450). The compound will also be tested in neuromyeltis optica, multiple sclerosis and as an add-on therapy in patients suffering from central neuropathic pain (NCT03138421). It will be interesting to see whether MAGL inhibitors mimic some of the psychoactive effects of cannabinoid CB1 receptor agonists, such as Δ^9-THC, the psychoactive component in marijuana, or whether chronic dosing leads to functional antagonism of the CB1 receptor.[35, 38]

1.4.2 PF-06795071

PF-06795071 is a potent and selective irreversible MAGL inhibitor which was reported by Pfizer.[14] This compound contains a carbamate warhead, [3.1.0] pyrazole core system and unique trifluoromethyl glycol leaving group (Figure 4). PF-06795071 showed high MAGL inhibitory activity (with an IC_{50} of 3 nM) without significant inhibition of other serine hydrolases and CB receptors, with the exception of carboxyesterase 1 (CES1), which is inhibited at 80% when tested at 10 μM. Moreover, it showed absence of binding at the hERG channel (IC_{50} > 30 μM), suggesting a low risk of cardiovascular QT prolongation. PK/PD studies showed that administration of PF-06795071 (1 mg/kg, subcutaneous) increased in brain 2-AG levels, which persisted for 8 h post-dose, and decreased in brain AA level over a similar period. PF-06795071 demonstrated potent anti-neuroinflammation effects in LPS-induced sepsis or encephalitis-like state where it significantly reduced levels of brain inflammatory markers (PGE2, IL-1β and TNF-α). So far, clinical trial studies have not been performed for this compound.

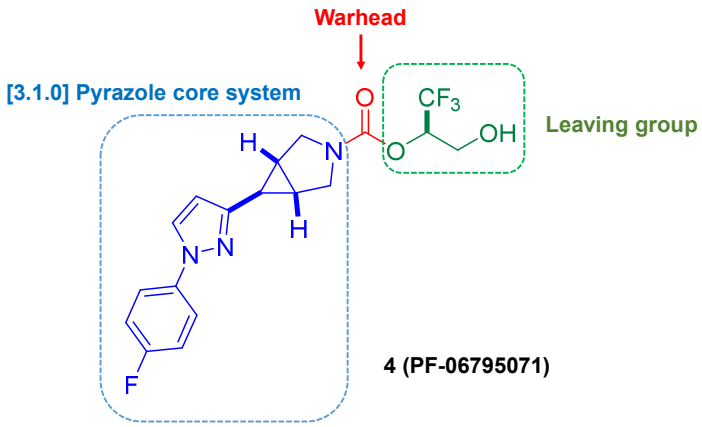

Figure 4. Chemical structure of PF-06795071.

1.4.3 Reversible MAGL inhibitors

Irreversible inhibitors may have several disadvantages to act as therapeutics. The reactive warhead might reduce the selectivity and induce idiosyncratic drug-related toxicity. In the case of MAGL inhibition, chronic exposure to irreversible MAGL inhibitor resulted in pharmacological tolerance.[52, 53] Reversible inhibitors may provide a chance to avoid these undesirable side-effects. Several series of reversible MAGL inhibitors have been patented or published by different pharmaceutical companies.[27, 50] All those compounds are amide-based MAGL inhibitors. In 2010, Johnson & Johnson patented a series of azetidine derivatives as reversible MAGL inhibitors[54] and crystallography studies demonstrated the reversible binding mode for one of those compounds (compound **5**, Figure 5).[55] The amide moiety of compound **5** formed two hydrogen bounds with Ala51 and Met123 of MAGL and no covalent bound between MAGL and the ligand was observed. The subsequent development led to the discovery of compound **6**. Compound **6** showed an IC_{50} < 5 nM. Mice administered with compound **6** (30 mg/kg, p.o.) showed less food consumption than control mice over a period of 30 min. Moreover, oral administration of this compound at doses of 0, 15, and 50 mg/kg/day for five consecutive days resulted in a decrease of mean body weight at the maximum tested concentration. Finally, *in vivo* studies were performed in dogs, which were subjected to a similar treatment, with doses of 0, 5, 15, and 45 mg/kg/day for five consecutive days. Treated dogs showed a decrease in body weight and food consumption, thus confirming the experimental results observed in mice.[50] In 2013, the

same company filed another patent reporting a series of compounds with similar structure of compound **6**, mainly differing for the presence of a piperidine instead of the piperazine ring.[54] The representative compounds **8a** and **8b** showed IC_{50} values on MAGL lower than 5 nM.

In recent years, Takeda Pharmaceutical company reported piperazinyl pyrrolidin-2-one derivatives as reversible MAGL inhibitors. The best compound (compound **9**, Figure 5) in this series was developed starting from pyrrolidinone hit compounds using a structure-based drug design (SBDD) approach.[27] Compound **9** showed high MAGL inhibitory activity with an IC_{50} of 3.6 nM. It showed high selective over FAAH (IC_{50} > 1000 nM), however, selectivity profiles over other serine hydrolases like ABHD6 and ABHD12 are still not reported. PK studies demonstrated that high exposure level of compound **9** was observed in both plasma and brain (dose 10 mg/kg; plasma concentration 1.01 μg/mL; brain concentration 0.656 μg/g) after 1 h of oral administration to mice. PD studies showed that oral administration of compound **9** significantly reduced AA levels (25%) and increased 2-AG levels (340%) in mouse brain.

Besides, Hoffmann-La Roche recently filed several patents describing reversible MAGL inhibitors.[56-58] However, limited information is available for those compounds at this moment.

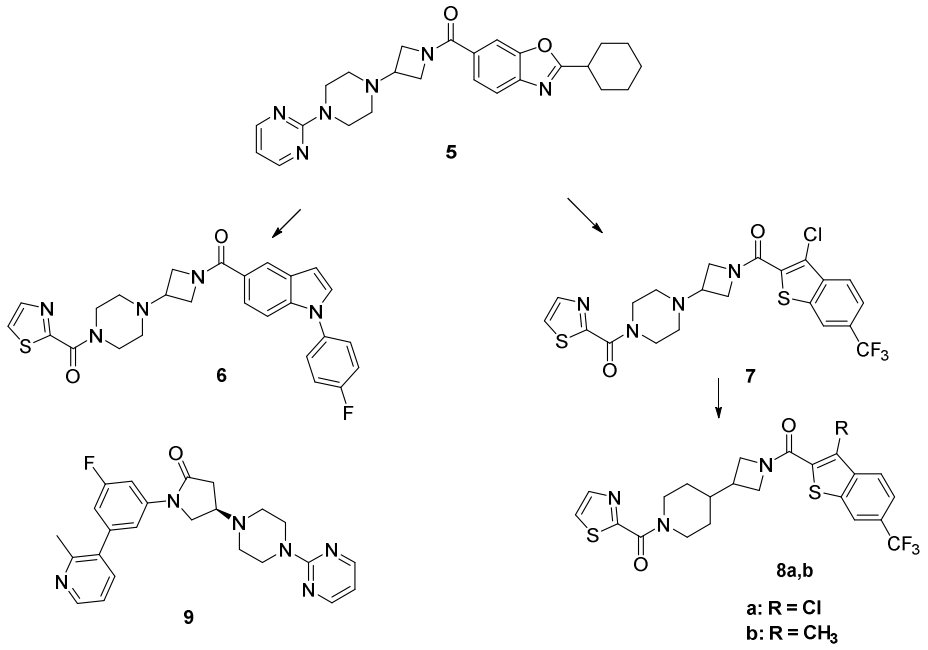

Figure 5. Chemical structures of reversible MAGL inhibitors developed by Johnson & Johnson and Takeda.

1.5 Aim and outline of this thesis

In view of the advantages of a reversible MAGL inhibitor, the aim of the research in this thesis is to develop potent, selective, reversible and *in vivo* active MAGL inhibitors. Previously, a high-throughput screening was performed in Pivot Park Screening Centre and a suitable starting point was identified.[59] In this thesis, the hit compound was further optimized which led to the discovery of novel chemotypes for MAGL inhibition. The outline of this thesis is as follows:

Chapter 2 describes the activity-driven optimization of a new hit compound of MAGL inhibitor found in a previously reported high-throughput screening campaign.[59] Ligand-based drug design approaches were carried out during the optimization progress, which led to the discovery of β-sulfinyl esters as highly potent and selective MAGL inhibitors.

Chapter 3 discusses the structure-activity relationship of α-aryl ketones as novel MAGL inhibitors which might have a covalent, reversible mode-of-action.

Chapter 4 focusses on the metabolic stability-guided optimization of β-sulfinyl esters towards drug-like MAGL inhibitors. *In vitro* metabolic stability assays using liver S9 fractions were performed to estimate the hepatic clearance rates of the new MAGL

inhibitors, which led to the discovery of LEI-515 as ultrapotent and metabolically stable MAGL inhibitor.

Chapter 5 describes the profiling of LEI-515 in biochemical, cellular and ADME-T assays as well as mouse pharmacokinetic and target engagement studies.

Chapter 6 summarizes the work presented in this thesis, and shows future directions for the disclosed research.

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