Discovery of reversible monoacylglycerol lipase inhibitors

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Chapter 6

Summary and future prospects

The aim of the research described in this thesis was to develop potent, selective and reversible monoacylglycerol lipase (MAGL) inhibitors that can be used as chemical tools to study MAGL function *in vivo* and further developed as therapeutics for the treatment of inflammation and cancer.

Chapter 1 provided an overview of the function and mode-of-action of MAGL. The enzyme is a membrane-associated protein belonging to the large family of serine hydrolases. It contains a typical α/β hydrolase fold and employs a Ser-His-Asp catalytic triad for the hydrolysis of monoacylglycerides into free fatty acid and glycerol.[1, 2] MAGL is the key enzyme involved in the degradation of the endogenous signaling lipid 2-arachidonoylglycerol (2-AG) (Figure 1).[3] 2-AG is an endocannabinoid which activates cannabinoid receptors.[4, 5] Activation of cannabinoid receptors by endogenous ligands plays an important role in various physiological processes, such as learning and memory, pain sensation, energy balance and inflammation.[6-8] Besides, MAGL is the predominant enzyme regulating the production of arachidonic acid (AA) in the brain, lung and liver. AA is the precursor of pro-inflammatory prostaglandins.[2] Therefore, MAGL inhibition is thought to have several therapeutic applications, such as anti-

inflammation, antinociception and anti-cancer. Of note, MAGL inhibitors might avoid gastrointestinal and cardiovascular side effects observed with dual cyclooxygenase 1/2 (COX1/2) and selective COX2 inhibitors because MAGL only controls eicosanoid metabolism in specific tissues.[9-11]

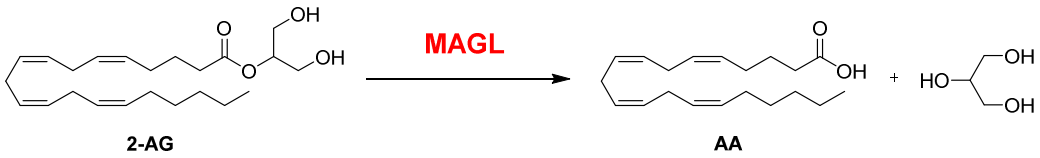

Figure 1. MAGL is the main enzyme for the hydrolysis of endocannabinoid 2-AG.

A number of drug discovery programs have been initiated to discover potent and selective MAGL inhibitors in the past two decades. Both irreversible and reversible MAGL inhibitors have been reported or patented (Figure 2).[12-15] The first-in-class MAGL inhibitor ABX-1431 (**2**, Figure 2), an irreversible MAGL inhibitor, is now in clinical trial phase 1b for the treatment of posttraumatic stress disorder (NCT04597450).[13] Irreversible inhibitors may have several advantages to act as therapeutics, like increased potency, long residence time and a less stringent pharmacokinetic profile.[16] However, the irreversible mode of action may also have some drawbacks, such as reduced selectivity, idiosyncratic drug-related toxicity and, in case of MAGL inhibition, pharmacological tolerance.[17, 18] Careful dosing or reversible inhibitors may avoid these unfavorable side-effects.[19]

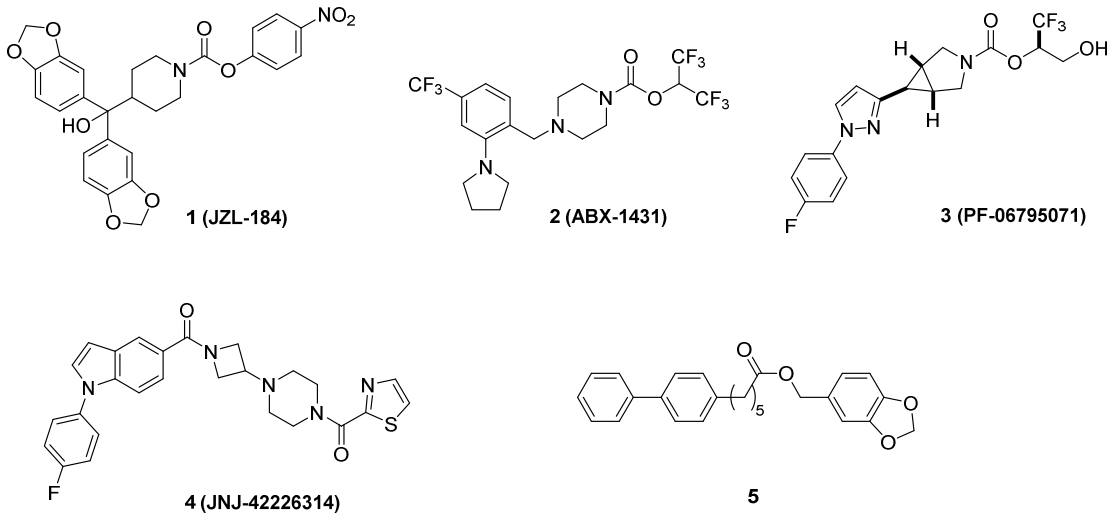

Figure 2. Chemical structures of reported MAGL inhibitors.

Previously, a high-throughput screen (HTS) was performed at the Pivot Park Screening Centre and seven compounds were validated as confirmed hits.[20] In **Chapter 2**, the optimization of β-sulfinyl ester-based hit **1** (Figure 1) as a novel chemotype for MAGL inhibition is described. A natural substrate-based MAGL activity assay was used to guide the hit optimization. The assay utilizes an enzymatic cascade to convert glycerol, a metabolite produced by MAGL, into a fluorescent signal.[21] A ligand-based drug design approach was performed to improve the potency of hit **6** (Figure 3). 56 compounds were synthesized and tested. This led to the discovery of compound **± 7** (Figure 3) as a potent MAGL inhibitor with single digital nanomolar potency (pIC_{50} = 8.50 ± 0.10). The selectivity of compound **± 7** was profiled by using activity-based protein profiling (ABPP) and it showed high selectivity against a panel of serine hydrolases, including α, β-hydrolase domain-containing protein 6 (ABHD6), ABHD12, diacylglycerol lipases (DAGLs) and fatty acid amide hydrolase (FAAH).

Compound **± 7** contains an ester functionality, which is a metabolic liability and may act as an electrophile for the incoming catalytic serine of MAGL. To test the latter hypothesis, the replacement of the ester group with an activated ketone as reversible, covalent warhead is described in **Chapter 3.** A series of 21 α-aryl ketones was designed, synthesized and tested, which led to the discovery of compound **± 8** (Figure 3) as a novel ketone-based MAGL inhibitor with an IC_{50} of 10 nM. This is the first alpha-keto heterocycle described as MAGL inhibitor.

In **Chapter 4**, the metabolic stability of compound **± 7** was evaluated using a liver S9-based assay. Compound **± 7** showed low metabolic stability as expected, because it contains a metabolically labile ester functionality. To optimize the metabolic stability of compound **± 7**, three different strategies were employed to improve the metabolic stability: 1) reducing the lipophilicity; 2) applying steric hindrance and 3) replacing the ester group with bioisosteres. Replacing the ester group with α-CF_2 ketone group led to the discovery of **LEI-515** (**9**, Figure 3) as a potent and metabolically stable MAGL inhibitor with subnanomolar potency.

The overall structure-activity relationship (SAR) for MAGL inhibitors developed in this thesis is displayed in Figure 3B.

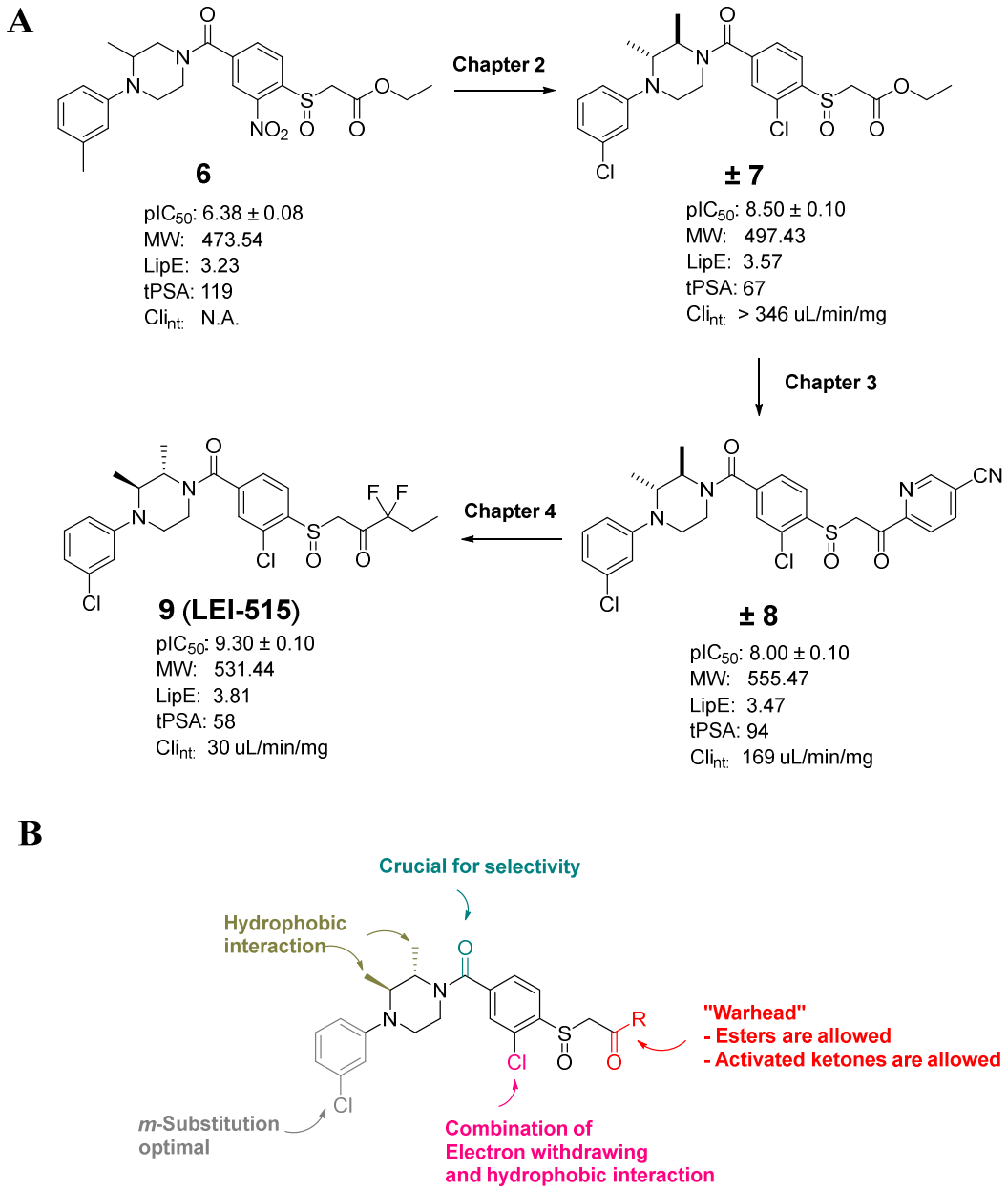

Figure 3. (**A**) Chemical structures, physical chemical properties and biological properties of hit compound (**6**) and analogues with improved potency (**± 7 - 9**). (**B**) The overall structure-activity relationship.

In **Chapter 5**, **LEI-515** was further profiled in biochemical, cellular and ADME assays as well as mouse pharmacokinetic and target engagement studies to assess its ability to act as a reversible and *in vivo* active MAGL inhibitor. Crystallography studies showed that **LEI-515** occupied the active site of MAGL and bound to the catalytic Ser122 covalently. The formed deprotonated hemiketal was stabilized by two

hydrogen-bounds with Ala51 and Met123, respectively (Figure 4). Competitive ABPP with tailor-made MAGL probe suggested that **LEI-515** is a reversible MAGL inhibitor. Activity-based protein profiling with broad-spectrum fluorophosphonate-based and β-lactone-based probes revealed that **LEI-515** was a highly selective MAGL inhibitor. **LEI-515** did not affect other enzymes involved in endocannabinoid metabolism, including ABHD6, ABHD12, DAGLs and FAAH. Hormone-sensitive lipase (LIPE) was identified as an off-target in mouse brain, liver and lung proteome. In vitro pharmacological profiling demonstrated that LEI-515 is selective (>100-fold selectivity) over a panel of 44 ion channels, receptors and enzymes, including the cannabinoid receptors (CB1R and CB2R), hERG channel and cyclooxygenases (COX1 and COX2). Targeted lipidomics revealed that **LEI-515** increased 2-AG levels in time- and concentration-dependent manner in human breast cancer HS578t cells. **LEI-515** also reduced the AA and AEA levels in the cells at 1 μM. Cell viability assay showed that **LEI-515** impaired the cells growth of 15 colorectal cancer cell lines with concentration for 50% of maximal effect (EC_{50}) in the range from 2 to 20 μM. Absorption, distribution, metabolism and excretion (ADME) profiling showed that **LEI-515** possess high stability (100 % remaining at 180 min) in both human and mouse plasma. Clearance in human microsomes (30.9 μL/min/mg) was moderate and low in mouse microsomes (< 3.4 μL/min/mg). **LEI-515** exhibited high protein binding (99.6% for both) in human and mouse plasma and showed negligible cell permeability in caco-2 cells ($P_{appA\text{-}B} < 0.01 \times 10^{-6}$ cm/s and $P_{appB\text{-}A} < 0.005 \times 10^{-6}$ cm/s). Pharmacokinetic study revealed excellent bioavailability and quick absorption of **LEI-515** after oral administration in mouse. Of note, **LEI-515** was found as a peripherally restricted MAGL inhibitor. Unfortunately, preliminary *in vivo* efficacy studies showed that **LEI-515** (10 mg/kg, p.o., 1h) did not increase 2-AG levels in the lung or liver, which might be due to high protein binding of the compound, too low dose and/or insufficient exposure time.

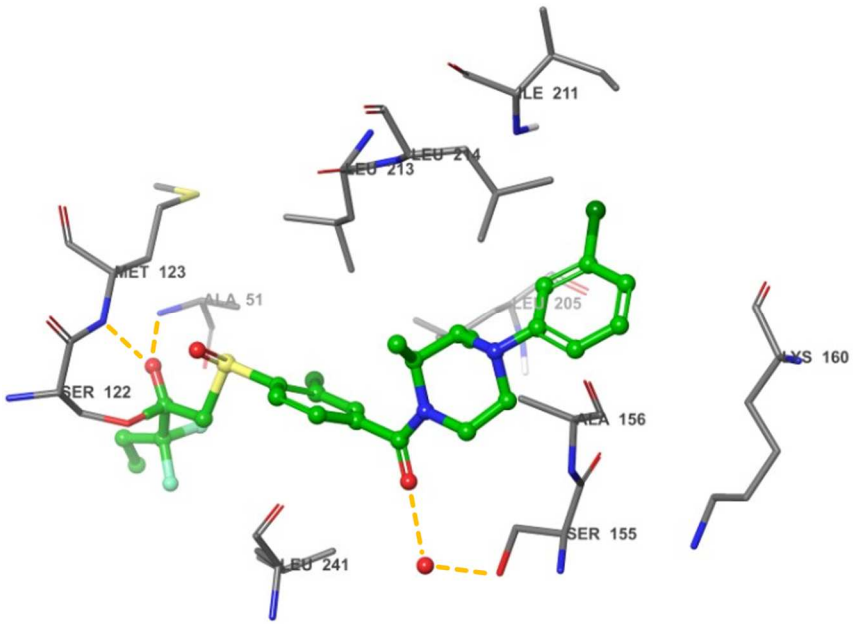

Figure 4. X-ray cocrystal structure of LEI-515 bound to hMAGL.

Future prospects

LEI-515 was discovered as a potent, selective, reversible and peripherally restricted MAGL inhibitor. Since **LEI-515** showed high protein binding and low *in vivo* efficacy, further optimization may be needed to reduce the lipophilicity of **LEI-515**. As it is known that free hydroxyl groups at the ethyl side are tolerated, compounds **± 10** to **± 12** (Figure 5) may be interesting novel MAGL inhibitors with lower lipophilicity.

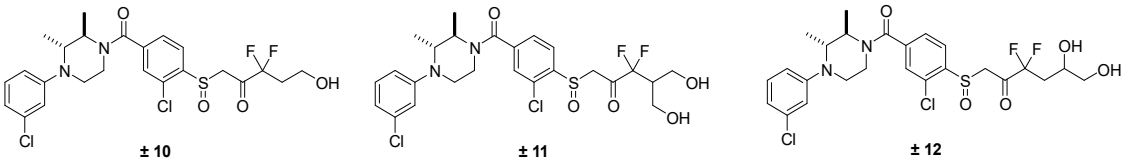

Figure 5. Proposed MAGL inhibitors with lower lipophilicity (**± 10** to **± 12**)

LEI-515 is the first peripherally restricted MAGL inhibitor with subnanomolar potency to date. It could be a useful tool compound to study MAGL function in peripheral tissues without disturbing the MAGL activity in the CNS. Higher dosing (*e.g.,* 30 mg/kg, p.o.) or longer exposure time (*e.g.* 2 h) may achieve sufficient *in vivo* MAGL inhibition for **LEI-515**. It has been revealed that MAGL inhibition exerted protective effects in lung and liver injury models.[22-24] Therefore, it would be interesting to study **LEI-515** in various animal models of human disease, such as hepatic

ischemia/reperfusion (I/R), lung I/R or, perhaps Covid-19 induced lung inflammation. Moreover, pharmacological and genetical inhibition of MAGL reduced tumor growth in several xenograft models, such as ovarian, melanoma, colon and prostate xenograft models.[25-27] It would also be interesting to evaluate the potential application of **LEI-515** as anti-cancer agent. In summary, this thesis described the discovery and optimization of the first reversible, peripherally restricted MAGL inhibitors based on activated ketones, which can be used to validate MAGL as a potential therapeutic target for various indications.

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