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The discovery of diazetidinyl diamides as potent and reversible inhibitors of monoacylglycerol lipase (MAGL)

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ABSTRACT

Monoacylglycerol lipase (MAGL) has emerged as an attractive drug target because of its important role in regulating the endocannabinoid 2-arachidonoylglycerol (2-AG) and its hydrolysis product arachidonic acid (AA) in the brain. Herein, we report the discovery of a novel series of diazetidinyl diamide compounds **6** and **10** as potent reversible MAGL inhibitors. In addition to demonstrating potent MAGL inhibitory activity in the enzyme assay, the thiazole substituted diazetidinyl diamides **6d–l** and compounds **10** were also effective at increasing 2-AG levels in a brain 2-AG accumulation assay in homogenized rat brain. Furthermore, selected compounds have been shown to achieve good brain penetration after oral administration in an animal study.

Monoacylglycerol lipase (MAGL) is a serine hydrolase that is primarily responsible for degrading 2-arachidonoylglycerol (2-AG) to arachidonic acid (AA) and glycerol in the brain.[1] 2-AG is a prominent endocannabinoid and an agonist for both cannabinoid receptor 1 (CB1) and cannabinoid receptor 2 (CB2).[2] AA, the 2-AG hydrolysis product by MAGL, is a precursor for proinflammatory eicosanoids that can cause neuroinflammation and lead to neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease.[3] Inactivation of MAGL has been shown in animal models to elevate the level of 2-AG and reduce the level of AA in the brain.[4] Consequently, MAGL inhibition potentially brings a dual benefit in treating diseases with an inflammatory component, first by increasing 2-AG mediated cannabinoid receptor activation and second by reducing the supply of AA-derived proinflammatory eicosanoids.[5] Furthermore, MAGL has been implicated in certain cancer cell growth; therefore, MAGL inhibitors may also be useful as anti-cancer agents.[5,6] As a result, MAGL has gathered increasing attention as an attractive drug target, and a number of MAGL inhibitors have been reported. The majority of those reported inhibitors, however, are irreversible and form a covalent bond with MAGL;[5c,7] and studies have shown that chronic inhibition of MAGL by an irreversible MAGL inhibitor or genetic deletion of MAGL leads to desensitization of CB1, which eventually impairs the beneficial 2-AG mediated effects that are CB1-dependent.[4a,4b] A potential approach to alleviate such drawbacks associated with irreversible inhibition of MAGL is to identify reversible MAGL inhibitors for therapeutic use.[5c,8]

We have previously reported a potent reversible MAGL inhibitor (compound **1**, Fig. 1).[9] Structural optimization based on compound **1** to improve metabolic stability led to the discovery of a novel series of diazetidinyl diamides (Fig. 1) as potent reversible inhibitors of MAGL.

The synthesis of diazetidinyl diamide compounds **6** is shown in Scheme 1. 1-Boc-3-amino-azetidine **2** was reacted with carboxylic acid (R^1CO_2H) or acid chloride (R^1COCl) under standard amide formation conditions to give compound **3**. Removal of the Boc protecting group under acidic conditions (CF_3CO_2H, CH_2Cl_2) followed by reductive amination with 1-Boc-3-azetidinone **4** [$Na(OAc)_3BH$, acetic acid, 1,2-dichloroethane] led to compound **5**. The Boc protecting group of **5** was removed (CF_3CO_2H, CH_2Cl_2), and the resulting product was reacted with a second carboxylic acid (R^2CO_2H) or acid chloride (R^2COCl) to give the final product diazetidinyl diamide **6**.

Representative diazetidinyl diamide compounds **6** are summarized in Table 1. *In vitro* MAGL inhibitory activity of the compounds was measured in an enzyme assay using purified wild-type human MAGL or mutant human MAGL,[10] which is a more soluble form of the wild-type enzyme with comparable catalytic activity.[9] In addition, a brain 2-AG accumulation assay was established to assess a given compound's effectiveness in elevating 2-AG levels in rat brain tissue, a consequence of MAGL inhibition.[11] In this assay, 2-AG levels in homogenized rat brain incubated with test compound were measured and compared to those incubated with the vehicle. The activity of the test compound is expressed as percent brain 2-AG accumulation, which is indicative of a compound's effectiveness at inhibiting MAGL in brain. Higher brain 2-AG accumulation (%) indicates a more effective inhibitor.

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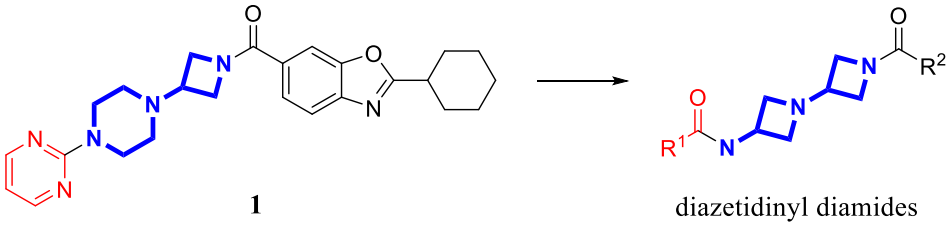
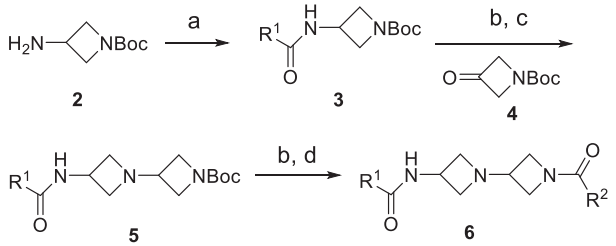


Fig. 1. Design of diazetidinyl diamides as reversible MAGL inhibitors.



Scheme 1. a) R^1CO_2H, HATU, Et_3N, CH_2C1_2; or R^1COC1, Et_3N, CH_2C1_2; b) TFA, CH_2C1_2; c) 4, $Na(OAc)_3BH$, HOAc, 1,2-dichloroethane; d) R^2CO_2H, HATU, Et_3N, CH_2C1_2; or R^2COC1, Et_3N, CH_2C1_2.

As illustrated by the data in Table 1, thiazole as the R^1 group led to compounds with much higher potency in the MAGL enzyme assay than the corresponding analogs where the R^1 group is phenyl (**6d**, **6e, 6h** vs **6a–c**). The 2-thiazole and 4-thiazole R^1 groups were not substantially different (**6f**, **6g, 6i** vs **6j–l**). When the R^1 group was 2-thiazole, analogs with fused heteroaromatic R^2 groups such as 2-phenyl benzoxazole (**6e**), *N*-phenyl-indole (**6g**), and benzothiophene (**6h**, **6i**), as well as analogs with bicyclic aromatic R^2 groups such as benzylphenyl (**6d**) and 3′-CF_3-biphenyl (**6f**) exhibit good potency in the MAGL enzyme assay (IC_{50}) and in the brain 2-AG accumulation assay (Brain 2-AG Accumulation%).

The crystal structure of compound **6d** binding with mutant human MAGL (hMGL 1-303 K36A, L169S, L176S) was obtained (Fig. 2), which confirmed noncovalent binding of compound **6d** to MAGL.[12] Compound **6d** occupies the same binding pocket in MAGL as compound **1**, which is located between helices α4, α5, α6 and α7.[9] Similar as compound **1**, the azetidine-amide carbonyl of compound **6d** points into the oxyanion hole of MAGL and forms a hydrogen bond with the backbone amide NH of Met123 that is adjacent to the catalytic Ser122. In addition, the thiazole-amide carbonyl of compound **6d** makes a hydrogen bond to the sidechain guanidine group of Arg57, and there is a π-π stacking interaction between the thiazole-amide and Tyr194 (Fig. 3).

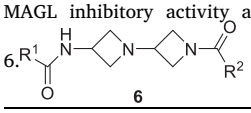
Because compounds **6a–c** (R^1 = phenyl) showed weak MAGL inhibitory activity and to further explore heterocyclic/heteroaromatic substitution at R^1, the related cyclic amide analogs **10** were synthesized (Scheme 2) and evaluated. Reductive amination [$Na(OAc)_3BH$, HOAc, 1,2-dichloroethane] of methyl 2-formylbenzoate **7** with 1-Boc-3-aminoazetidine **2** followed by spontaneous lactam formation gave the cyclized amide intermediate **8**. Deprotection under acidic conditions (CF_3CO_2H, CH_2Cl_2) removed the azetidine Boc group, and a second reductive amination with 1-Boc-3-azetidinone **4** [$Na(OAc)_3BH$, acetic acid, 1,2-dichloroethane] led to compound **9**. Removal of the Boc protecting group of **9** (CF_3CO_2H, CH_2Cl_2) followed by an amide formation reaction with carboxylic acid (R^2CO_2H) or acid chloride (R^2COCl) resulted in the final product diazetidinyl diamide **10**.

Results for a selection of compounds **10** are summarized in Table 2. The data indicate compounds **10** achieve a significant improvement of MAGL inhibitory activity compared to compounds **6** of which R^1 = phenyl (**10a** vs **6b**). The compounds **10** illustrated in Table 2 demonstrate similar potency to compounds **6** with R^1 = 2-thiazole in Table 1, considering both MAGL enzyme activity and brain 2-AG accumulation (**10a**, **10c**, **10e** vs **6e**, **6g**, **6i**).

To assess their selectivity for MAGL, compounds **6e**, **6f** and **10e** were tested against fatty acid amide hydrolase (FAAH), and all three compounds showed no significant inhibitory activity (FAAH IC_{50} > 10 μM). Additionally, based on their *in vitro* potency and ADME properties, several compounds were selected to be evaluated in a rat tissue distribution study (10 mg/Kg, po) for their ability to penetrate the blood–brain barrier (BBB). Blood and brain samples were collected at the 1 h time point and analyzed. Among the 4 compounds in Table 3, compound **10f** achieved the highest brain concentration (1.20 nmol/g), while compound **6f** showed the highest brain/plasma ratio (0.85).

In summary, a novel series of diazetidinyl diamide compounds were discovered as potent reversible MAGL inhibitors. The thiazole

Table 1
MAGL inhibitory activity and 2-AG accumulation of selected compounds



Cpd	R^1	R^2	MAGL IC_{50} (nM)	Brain 2-AG accumulation (%) (Cpd conc.)
1			10[a]	N/A
6a			270[a]	N/A
6b			939[a]	N/A
6c			458[a]	N/A
6d			15[a]	659% (10 μM)
6e			48[a]	486% (10 μM) 355% (1 μM)
6f			7[a]	921% (10 μM) 347% (1 μM)
6g			12[b]	338% (1 μM)
6h			28[a]	611% (10 μM)
6i			< 5[a]	460% (1 μM)
6j			6[a]	620% (1 μM)
6k			15[a]	220% (10 μM)
6l			< 5[a]	477% (1 μM)

[a] Mutant MAGL.
[b] Wild-type MAGL; N/A: Data not available.

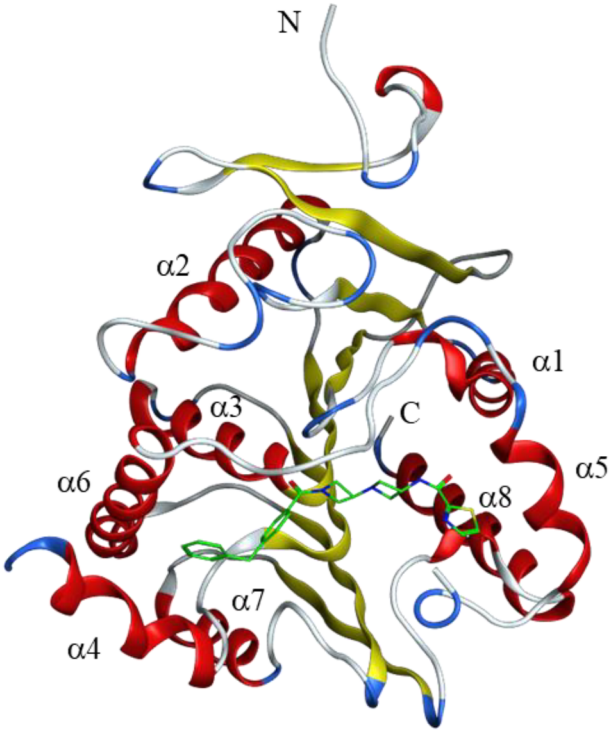


Fig. 2. Overview of the cocrystal structure of compound 6 d (green) and mutant human MAGL.

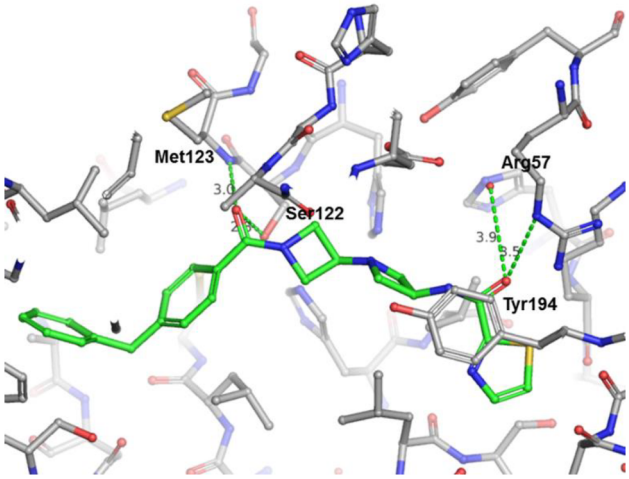
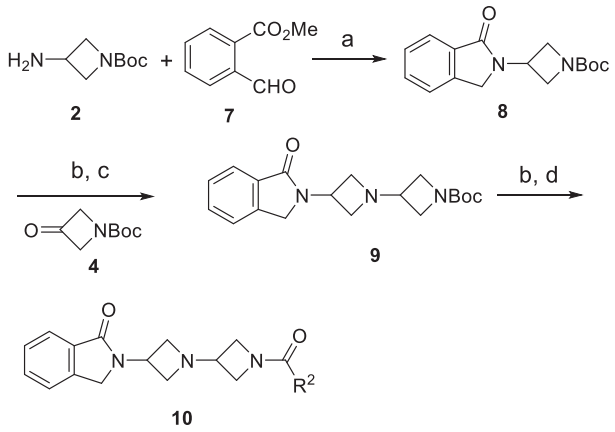


Fig. 3. A close-up view of the compound 6d (green) binding site to MAGL.

substituted compounds **6d–l** (R^1 = thiazole) exhibited high MAGL inhibitory potency. Structural modification of the less potent phenyl substituted compounds **6a–c** (R^1 = phenyl) led to a series of cyclic amide analogs **10** with much more improved MAGL inhibitory activity. Selected compounds also demonstrated good brain penetration in rat after an oral dose. Such compounds may serve as tools for future research of MAGL inhibition as a therapeutic approach to treat disorders including pain, inflammation, and depression.

Declaration of Competing Interest

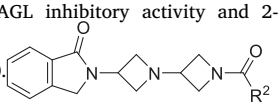
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.



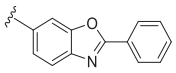
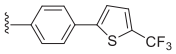
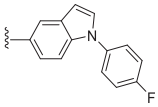
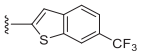
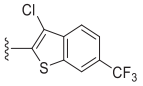
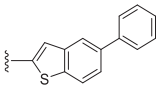
Scheme 2. a) $Na(OAc)_3BH$, HOAc, 1,2-dichloroethane; b) TFA, CH_2C1_2; c) 4, $Na(OAc)_3BH$, HOAc, 1,2-dichloroethane; d) R^2CO_2H, HATU, Et_3N, CH_2C1_2; or R^2COC1, Et_3N, CH_2C1_2.

Table 2
MAGL inhibitory activity and 2-AG accumulation of selected compounds

10.



10

Cpd	R^2	MAGL IC_{50} (nM)	Brain 2-AG Accumulation (%) (Cpd conc.)
10a		31[a]	435% (10 μM) 152% (1 μM)
10b		15[a]	725% (10 μM) 204% (1 μM)
10c		11[b]	332% (1 μM)
10d		19[a]	409% (10 μM) 192% (1 μM)
10e		< 5[a]	449% (1 μM)
10f		18[a]	676% (10 μM) 253% (1 μM)

[a] Mutant MAGL.
[b] Wild-type MAGL.

Table 3
Plasma and brain concentrations of selected compounds.

Cpd	Plasma conc. (μM)	Brain conc. (nmol/g)	B/P ratio
6e	2.17	0.84	0.39
6f	0.81	0.67	0.85
6j	1.08	0.35	0.33
10f	4.76	1.20	0.25

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10. All rate-based assays were performed in black 384-well polypropylene polymerase chain reaction ("PCR") microplates (Abgene) in a total volume of 30 μL. Substrate 4-methylumbelliferyl butyrate (4MU-B; Sigma) and purified mutant MAGL (mut-MGLL 11-313 L179S L186S) or wild type MGL (wt-MGLL 6H-11-313) were diluted separately into 20 mM 1,4-piperazinediethanesulfonic acid ("PIPES") buffer (pH = 7.0), containing 150 mM NaCl and 0.001% Tween 20. Test compounds were pre-dispensed (50 nL) into the assay plate using a Cartesian Hummingbird prior to adding 4MU-B (25 μL of 1.2X solution to a final concentration of 10 μM) followed by enzyme (5 μL of a 6X solution to a final concentration of 5 nM) to initiate the reaction. Final compound concentrations ranged from 17 to 0.0003 μM. The fluorescence change due to 4MU-B cleavage was monitored with excitation and emission wavelengths of 335 and 440 nm, respectively, and a bandwidth of 10 nm (Safire2, Tecan) at 37°C for 5 min. The IC50 values for the test compounds were determined using Excel from a fit of the equation to the concentration-response plot of the fractional activity as a function of inhibitor concentration.
11. One gram of rat brain was homogenized using a Polytron homogenizer (Brinkmann, PT300) in 10 mL of 20 mM HEPES buffer (pH = 7.4), containing 125 mM NaCl, 1 mM EDTA, 5 mM KCl, and 20 mM glucose. Test compounds (1 μM) or vehicle were pre-incubated with rat brain homogenate (50 mg). After a 15-min incubation time at 37°C, CaCl2 (final concentration = 10 mM) was added and then incubated for 15 min at 37°C in a total volume of 5 mL. The reaction was stopped with 6 mL organic solvent extraction solution of 2:1 chloroform/methanol. The concentration of 2-AG in the organic phase was measured by a HPLC/MS method, and Brain 2-AG Accumulation% of the compound was derived from the following formula.
12. MAGL construct (hMAGL 1-303, K36A, L169S, L176S mutant) in 50 mM HEPES buffer (pH 7.5) with 200 mM NaCl, 2% glycerol, 2 mM EDTA, and 2 mM dithiothreitol, was mixed with 2-fold excess of (3-iodo-4-methylphenyl)(3-(4-(pyrimidin-2-yl)piperazin-1-yl)azetidin-1-yl)methanone and incubated overnight at 4°C and was then concentrated to 5-6mg/mL for crystallization. Crystals were obtained by mixing 1 μL of complexed protein with 0.2 μL seed stock and 0.5 μL of 8–13% PEGMME 5000, 100 mM Na-Citrate (pH 5.5), 0.2% glucopyranoside and equilibrated against 1 mL reservoir of 6–11% PEGMME 5000, 100 mM Na-Citrate pH 5.5, 0.2% glucopyranoside in a hanging drop vapor diffusion experiment at room temperature. These co-crystals were then used for displacement soaks with compound 6d (5 mM, overnight soak). The resulting crystals were harvested, transferred briefly into cryoprotectant containing 16% PEGMME 5000, 100 mM Na-MES (pH 6.0), and 25% glycerol, and flash frozen in liquid nitrogen. A complete dataset was collected for both on a Rigaku M007HF generator at 100°K. Data was processed in d*trek and structure refined in PHENIX.