Supporting Information for

Original article

[^{18}F]MAGL-4-11 positron emission tomography molecular imaging of monoacylglycerol lipase changes in preclinical liver fibrosis models

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Supplementary method

Radiosynthesis of [^{18}F]MAGL-4-11 ((4-(1-(4-(3-(fluoro-^{18}F)pyrrolidin-1-yl)benzoyl)azetidin-3-yl)piperazin-1-yl)(thiazol-2-yl)methanone)

The general labeling procedure for [^{18}F]MAGL-4-11 formation was described previously[1]. The cyclotron-produced [^{18}F] F^- was separated from $H_2{}^{18}O$ using the Sep-Pak Accell Plus QMA Plus Light cartridge (Waters; Milford, MA, USA). The produced [^{18}F]F^- was eluted from the cartridge with a mixture of aqueous K_2CO_3 (4 mg in 200 μL DMSO) and a solution of 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8,8,8]hexacosane (Kryptofix222; 7.5 mg) in CH_3CN (200 μL), and transferred to a reaction vessel in the hot cell. After drying [^{18}F]KF solution at 120 °C for 30 min to remove water and CH_3CN, a solution of mesylate precursor (2.0 mg) in anhydrous DMSO (300 μL) was then added. The vessel was heated at 120 °C for 10 min, then diluted with HPCL mobile phase (500 μL), followed by injection into an HPLC column. HPLC purification was performed on an X Bridge Prep C18 column (10 × 250 mm, 5 μm) using a mobile phase of $CH_3CN/H_2O/Et_3N$ (30/70/0.1) at a flowrate of 5.0 mL/min. The radioactive fraction corresponding to the desired product was collected in a sterile flask, evaporated to dryness in vacuo, and reformulated in PBS containing 5% EtOH. The synthesis time was ca. 70 min from end of bombardment. Radiochemical and chemical purity were measured by an analytical HPLC (X Bridge Prep C18 column (4.6 × 250 mm, 5 μm) using a mobile phase of CH_3CN / H_2O + 0.1% Et_3N (30/70) at a flow rate of 1.0 mL/min. The product identity was confirmed by the co-injection with unlabeled MAGL-4-11. Radiochemical yield was 39.3 ± 13.7% (*n* = 5) decay-corrected based on [^{18}F]F^- with >99% radiochemical purity, and the molar activity was 111.8–328.7 GBq/μmol (3.03–8.87 Ci/μmol) (Fig. S3).

Table S1. Characteristics of the liver fibrosis patients

Pts ID	Fibrosis Grades	Age (yr)	Sex	Race	Ethnicity
Pts 1	F0	65	M	White	Non-Hispanic
Pts 2	F0	72	M	White	Non-Hispanic
Pts 3	F0	65	M	White	Non-Hispanic
Pts 4	F1	84	M	White	Non-Hispanic
Pts 5	F1	60	M	White	Non-Hispanic
Pts 6	F1	64	M	White	Non-Hispanic
Pts 7	F2	54	M	White	Non-Hispanic
Pts 8	F2	56	M	White	Non-Hispanic
Pts 9	F2	58	F	White	Non-Hispanic

Table S2 Primers used for qPCR.

Gene name	Forward sequence 5'-> 3'	Reverse sequence 5'-> 3'
CB1	CACCTTCCGCACCATCACCAC	GTCTCCCGCAGTCATCTTCTCTTG
DAGLα	AGAATGTCACCCTCGGAATGG	GTGGCTCTCAGCTTGACAAAGG
MAGL	CAAGGCCCTCATCTTTGTGT	ACGTGGAAGTCAGACACTAC
FAAH	CCCAGATGGAACATTACAGG	CAGGATGACTGGTTTTCAGG
NAPE-PLD	CACGGTAATGGTGGAAATGG	GTCCAGATGGTCATAGTGGTTG
18s rRNA	GGGAGCCTGAGAAACGG	GGGTCGGGAGTGGGTAATTT

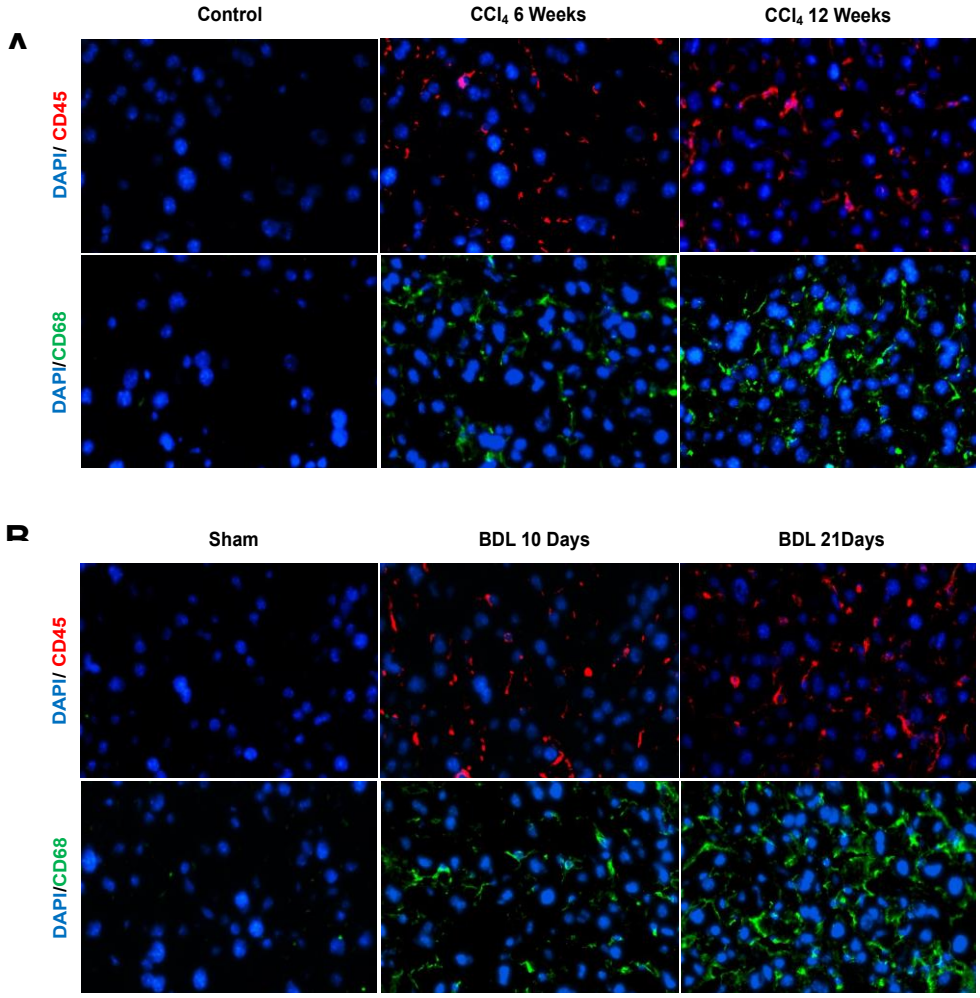


Figure S1 Hepatic panleukocyte (CD45) and macrophage (CD68) expression after CCl_4 (A) and BDL (B) treatment.

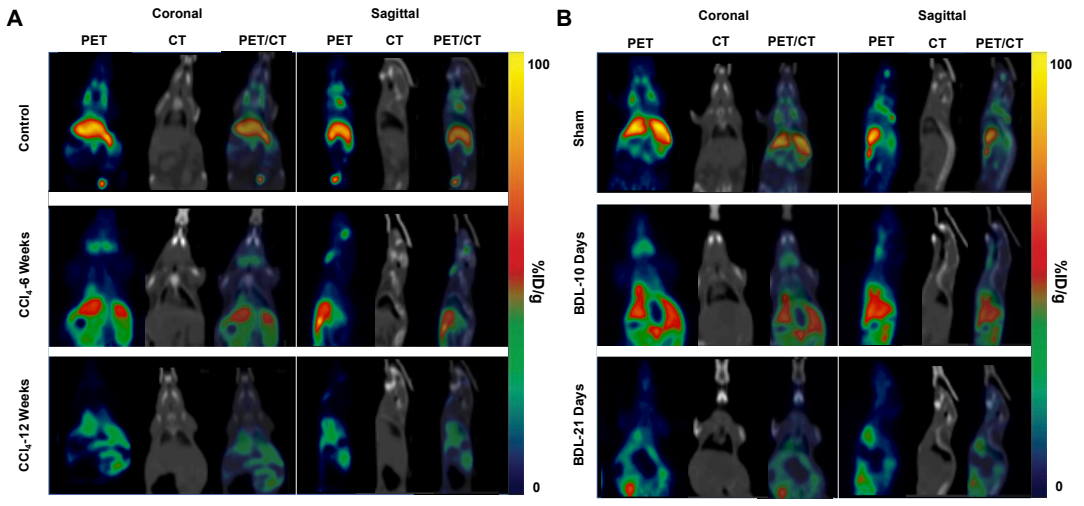
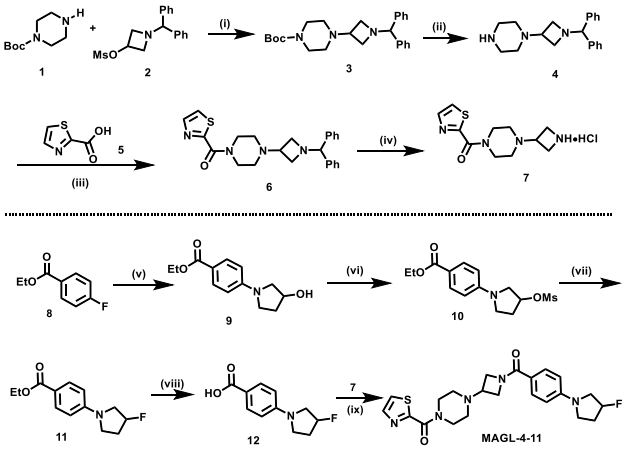
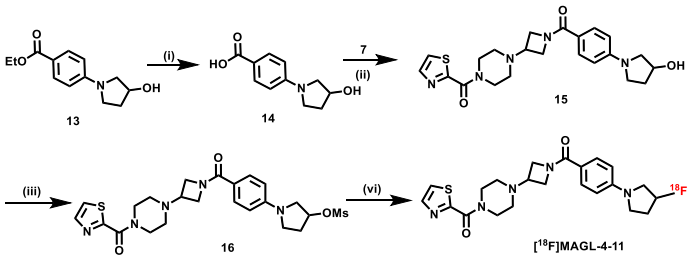


Figure S2 Coronal and sagittal images of [^{18}F]MAGL-4-11/PET-CT imaging hepatic MAGL expression with mild and severe CCl_4 (A) and BDL (B) mice.



Scheme S1 Synthesis of irreversible MAGL inhibitor (cold compound) MAGL-4-11. Conditions: (i) DIPEA, MeCN, 80 °C for 12 h; 85% yield; (ii) TFA, CH_2Cl_2, rt, 12 h; 99% yield; (iii) HOBT, EDC·HCl, Et_3N, DMF, rt, 12 h; 78% yield; (iv) 1-chloroethyl chloroformate, CH_2Cl_2, rt, 2 h; then MeOH, 35 °C 2 h; 86% yield; (v) pyrrolidin-3-ol hydrochloride, K_2CO_3, DMSO, 120 °C for 24 h; 79% yield; (vi) MsCl, Et_3N, CH_2Cl_2, rt, overnight; 84% yield; (vii) TBAF, THF, 70 °C, 2 h; 38% yield; (viii) LiOH, THF/MeOH/H_2O, 40 °C, 16 h; 92% yield; (ix) HOBT, EDC·HCl, Et_3N, DMF, rt, 12 h; 30% yield; DIPEA = *N*,*N*-diisopropylethylamine; TFA = trifluoroacetic acid; HOBT = 1-hydroxybenzotriazole hydrate; EDC·HCl = *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride; Et_3N = triethylamine; DMF = *N*,*N*-dimethylformamide; DMSO = methyl sulfoxide; MsCl = methanesulfonyl chloride; TBAF = tetrabutylammonium fluoride.



Scheme S2 Synthesis of MAGL PET tracer [^{18}F]MAGL-4-11. Conditions: (i) LiOH, THF/MeOH/H_2O, 40 °C, overnight; 97% yield; (iI) HOBT, EDC•HCl, Et_3N, DMF, rt, 12 h; 22% yield; (iii) MsCl, Et_3N, CH_2Cl_2, rt, overnight; 70% yield; (vi) [^{18}F]KF, DMSO, 120 °C, 10 min, 39% decay-corrected RCY. HOBT = 1-hydroxybenzotriazole hydrate; EDC·HCl = *N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride; DMF = *N*,*N*-dimethylformamide; DMSO = methyl sulfoxide; MsCl = methanesulfonyl chloride.

Reference

1. Chen Z, Mori W, Deng X, Cheng R, Ogasawara D, Zhang G, et al. Design, synthesis, and evaluation of reversible and irreversible monoacylglycerol lipase positron emission tomography (PET) tracers using a “tail switching” strategy on a piperazinyl azetidine skeleton. *J Med Chem* 2019;**62**:3336-53.