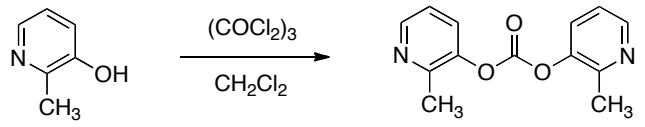
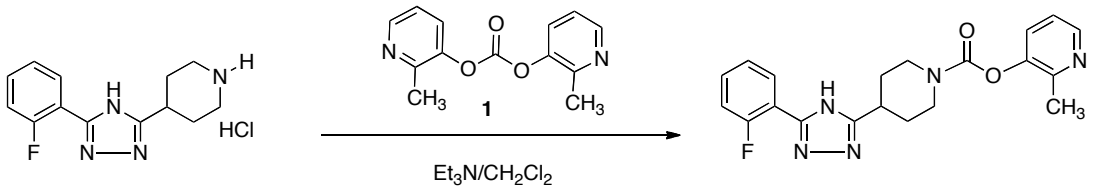
Supporting Information

Synthesis of bis(2-methylpyridin-3-yl) carbonate (1)



To a solution of triphosgene (300 mg, about 1.0 mmol), 3-hydroxy-2-methylpyridine (**2**; 655 mg, 6 mmol) in dichloromethane (50 mL) at 0°C, a solution of triethylamine (1.0 mL 7.5 mmol) in dichloromethane (10 mL) was added dropwise. The reaction mixture was stirred at room temperature overnight. This reaction was washed with brine and extracted with dichloromethane. The organic layer was washed with saturated $KHCO_3$ solution and brine, and dried with anhydrous sodium sulfate. The organic layer was evaporated to give a crude product, which was washed with *n*-hexane to give **1** as a white crystal (698 mg, 95.3%); mp: 75.5–76.5°C. ^{1}H–NMR (DMSO, δ) 8.44 (1H, d, J = 4.8 Hz), 7.86 (1H, d, J = 8.4 Hz), 7.37–7.42 (1H, m), 2.49 (3H, s).

Synthesis of 2-methylpyridin-3-yl-4-(5-(2-fluorophenyl)-4*H*-1,2,4-triazol-3-yl)piperidine-1-carboxylate) (MFTC)

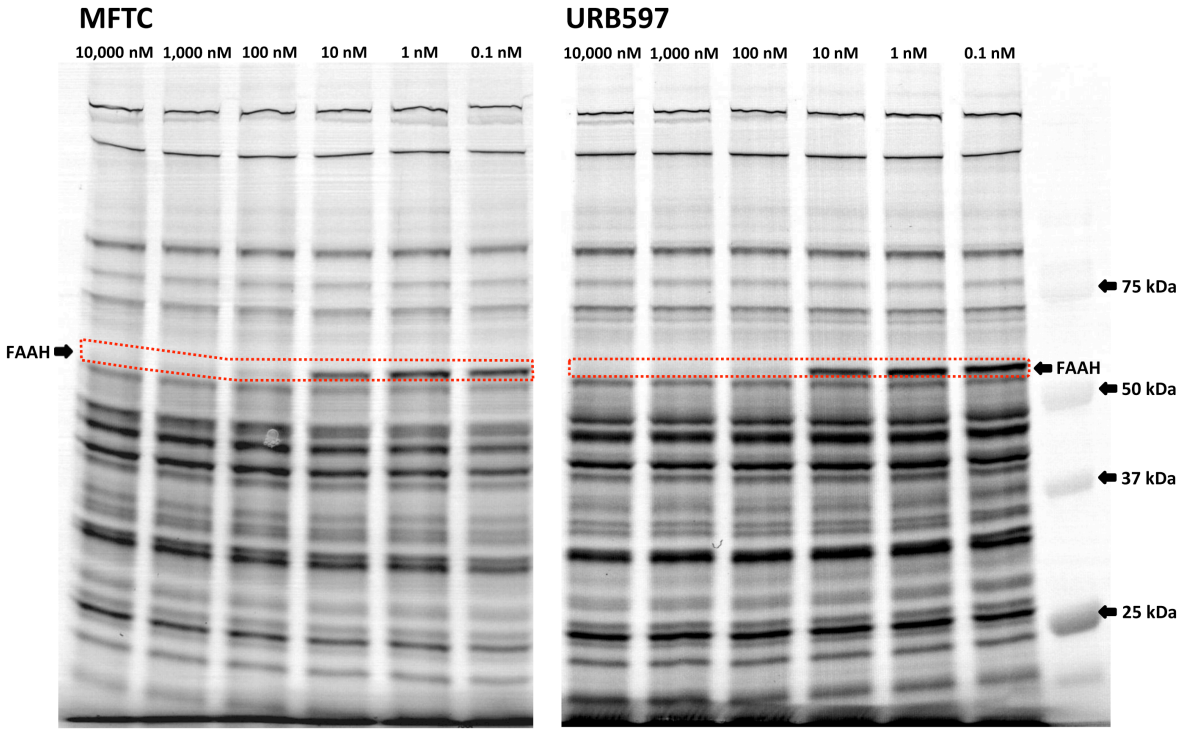


A mixture of 4-(5-(2-fluorophenyl)-4*H*-1,2,4-triazol-3-yl)piperidine hydrochloride (**3**•HCl; 70 mg, 0.248 mmol), bis(2-methylpyridin-3-yl) carbonate (**1**; 61 mg, 0.25 mmol), and triethylamine (70 μL, ca 0.5 mmol) in dichloromethane (5 mL) was stirred at room temperature overnight. The mixture was washed with brine and extracted with dichloromethane. The organic layer was washed with brine, dried with anhydrous sodium sulfate, and evaporated in vacuo. The obtained crude product was purified with silica gel chromatography (dichoromethan/methanol: 96/4) to give MTFC as a colorless solid (85.1 mg, 90.2%). ^{1}H-NMR ($CDCl_3$ δ): 8.38 (1H, d, J = 4.8 Hz), 8.25 (1H, J = 1.8 Hz, 7.6 Hz), 7.44–7.49 (2H, m), 7.15–7.34 (4H, m), 4.27-4.42 (2H, m), 3.10–3.31 (3H, m), 2.49 (3H, s),

2.02–2.22 (4H, m). FAB-MS $C_{20}H_{20}FN_5O_2$ 382 (M+1).

In vitro binding assay

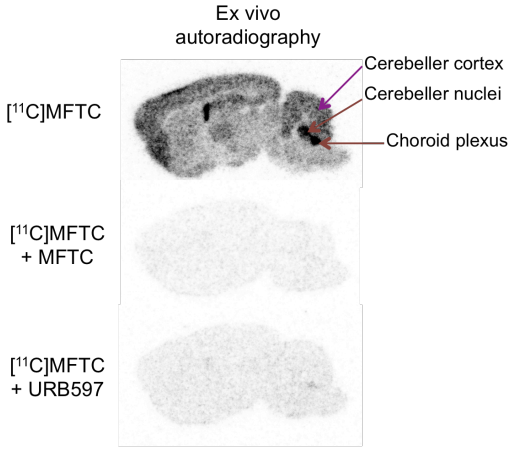
The binding affinity (IC_{50}) for FAAH was examined using competitive activity-based protein profiling. Membrane fractions of rat brain (50 μL, 1 mg/mL total protein concentration) were preincubated with varying concentrations of inhibitors at 37°C. After 30 min, a fluorophosphonate-rhodamine probe (1 μL, 50 μM in DMSO) was added and the mixture was incubated for another 30 min at 37°C. Reactions were quenched with SDS loading buffer, run on an SDS-PAGE gel, and visualized with a Fluor Image Analyzer FLA-5100 (GE Healthcare, Buckinghamshire, UK). Fluorescence of the relevant band at each inhibitor (MFTC and URB597) concentration was measured. Concentration-response curves were fit with GraphPad Prism 5 software (GraphPad Software, La Jolla, CA) to calculate the IC_{50} values.



Supplemental FIGURE 1. *In vitro* inhibition of FAAH by MFTC and URB597 as measured by competitive activity-based protein profiling. The gel images show concentration-dependent inhibition of FAAH by the two compounds in rat brain homogenates.

Ex vivo autoradiography

A saline solution of [^{11}C]MFTC (148 MBq, 2.5 nmol) was injected intravenously into rats. To investigate specific binding, the FAAH antagonist MFTC (1 mg/kg) or URB597 (3 mg/kg) was injected at 30 min before the radiotracer injection. At 30 min after the radiotracer injection, rats were sacrificed. The rat brain was removed and frozen on powdered dry ice. Brain sagittal sections (10 mm) were cut on a cryostat microtome (HM560; Carl Zeiss, Germany) and thaw-mounted on glass slides. These sections were then placed in contact with imaging plates (BAS-MS2025; FUJIFILM, Tokyo). Autoradiograms were obtained, and then photo-stimulated luminescence values (PSL) in regions of interest (ROIs) were determined using a Bio-Imaging Analyzer System (BAS5000; FUJIFLIM).



Supplemental FIGURE 2. Representative ex vivo autoradiographic images for [^{11}C]MFTC, [^{11}C]MFTC pretreated with MFTC (1 mg/kg), and [^{11}C]MFTC pretreated with URB597 (3 mg/kg) in rat brains.