SUPPORTING INFORMATION

A Novel ^{18}F-Labeled Imidazo[2,1-*b*]benzothiazole (IBT) for High-Contrast PET Imaging of β-Amyloid Plaques

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Experimental Section

General methods

All commercial reagents and solvents were used without further purification unless otherwise specified; compounds **3**-**6** were synthesized following the published procedure[1]. Microwave reactions were performed in microwave process vials with an Initiator EXP (Biotage, Uppsala, Sweden). LC/MS experiments were performed with an Ion-trap 500 Varian system with ESI (Varian Deutschland GmbH, Darmstadt, Germany) and NMR experiment done with Bruker Avence 360/500MHz. Analytical HPLC was performed using either a Chromolith RP18e (4.6 × 100 mm) eluted with a mixture of acetonitrile / 0.1 M ammonium formate (MeCN content between 27.5 and 50 %) at a flow rate of 5 mL/min or a Nucleosil 100, CN 4.6 × 250 mm reverse phase column, particle size 5 µm (CS-Chromatographie, Langerwehe, Germany) eluted with acetonitrile / 0.1 M ammonium formate (MeCN content between 40 and 60 %). Both chromatography systems were fitted with a UV detector (Sykam Model S3210 set at 254 nm; Sykam, Fürstenfeldbruck, Germany).

Syntheses procedure

2-(p-acetamidophenyl)-7-methoxyimidazo[2,1-b] benzothiazole **(3)**

A mixture of 6-methoxybenzo[d]thiazol-2-amine (180 mg, 1 mmol) and N-(4-(2-bromoacetyl)phenyl)acetamide (256 mg, 1 mmol) in 5 mL EtOH was heated overnight at reflux. The reaction mixture was cooled to room temperature, filtered, washed with 2 mL diethylether and the precipitate was dried under vacuum (318 mg, 94% yield, HPLC purity of >98%) ESI-MS, [M+1]=338.1; ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.07 (3H, s), 3.85 (3H, s), 7.19 (1H, *J* = 8.9, 2.6 Hz, dd), 7.66 (2H, *J* = 8.8 Hz, d), 7.71 (1H, *J* = 2.5 Hz, d), 7.75 (2H, *J* = 8.8 Hz, d), 7.93 (1H , *J* = 8.9 Hz, d), 8.68 (1H, s), 10.03 (1H, s) ; ^{13}C NMR (126 MHz, DMSO-d_6) δ 24.6, 56.4, 109.0, 109.9, 110.2, 114.6, 119.7, 125.6, 126.3, 127.9, 130.90, 139.3, 144.7, 146.6, 157.6, 168.7.

***2-(p-aminophenyl)-7-methoxyimidazo[2,1-b] benzothiazole* (4)**

3 (337 mg, 1 mmol), EtOH (10 mL) and 2 M NaOH (10 mL) were added in a microwave process vial capped with Teflon septum and sealed. The reaction mixture was microwave-irradiated under stirring at 100°C for 30 min. Then reaction mixture was cooled dawn to room temperature, concentrated under vacuum and to the residue 50 mL water was added and with CH_2Cl_2 (3 × 100 mL) extracted. The organic phase was washed with brine, dried over sodium sulphate and the solution concentrated. The crude product was purified by flash chromatography DCM: Methanol, the solvents evaporated under reduced pressure and the residue was dried under high vacuum (239 mg, 81% yield with HPLC purity of >98%) , LC-MS-ESI, [M+1] = 296.1; DMSO-d6 ^{1}H NMR δ 3.81 (3H,s), 5.16 (2H, s), 6.61 (2H,d), 7.11 (1H,dd) 7.48 (2H,d), 7.63 (1H,d), 7.82 (1H,d), 8.37 (1H, s); DMSO-d6 ^{13}C NMR δ 55.8, 106.2, 109.4, 113.5, 113.9, 121.9, 125.6, 130.2, 145.5, 147.2, 148.0, 156.6 and directly used for synthesis of **5**.

***2-(p-Methylaminophenyl)-7-methoxyimidazo[2,1-b] benzothiazole* (5)**

4 (239 mg, 0.81 mmol) was dissolved in DMF (10 mL) and treated with potassium carbonate (anhydrous, 2 eq., 280 mg). One equivalent of methyl iodide was added to the mixture at 90 °C. The reaction mixture was cooled to room temperature, 50 mL water was added, and the resulting mixture was loaded on RP flash chromatography cartridge and washed with 10 mL water. The crude product **5** was purified by flash chromatography. (108 mg, 43% yield with HPLC purity of >98%) ESI-MS, [M+1]= 310.1; ^{1}H NMR (DMSO-d6) δ 2.7 (3H, J= 5 Hz, d), 3.3 (3H, s), 5.7 (1H, br s), 6.6 (2H, J= 8.5 Hz, d), 7.1 (1H, JA= 8.5, JB= 2.5 Hz, q), 7.6 (4H, m), 8.4 (1H, s); ^{13}C NMR (DMSO-d6) δ 30.2, 56.3, 106.8, 109.9, 112.1, 114.1, 118.8, 122.2, 126.1, 126.6, 130.7, 146.1, 147.6, 149.7, 157.1.

***2-(p-Methylaminophenyl)-7-hydroxyimidazo[2,1-b] benzothiazole* (6)**

The compound **5** (93 mg, 0.3 mmol) was reacted with 1.5 molar equivalents of BBr_3 (1 molar solution) in 10 mL dichloromethane and microwave irradiation at 120°C for 30 min. The reaction

mixture was quenched by adding 1N HCl and extracted with 3 × 20 mL CH_2Cl_2. The combined organic phase was washed with saturated sodium bicarbonate solution (20 mL), dried over sodium sulphate and evaporated. The product (45 mg, 76% yield in >98% HPLC purity) was confirmed by LC-MS-ESI, [M+1] = 296.1; ^{1}H NMR (500 MHz, DMSO-d6) δ 2.70 (3H, s) , 3.33 (1H, s), 6.57 (2H, J = 9.1 Hz, d), 6.93 (1H, J = 2.9, 0.4 Hz, dd), 7.34 (1H, J = 2.4, 0.4 Hz, dd), 7.57 (2H, J = 8.7 Hz, d),7.72 (1H, J = 8.7, 0.4 Hz, dd), 8.33 (1H, s); ^{13}C NMR (126 MHz, DMSO-d6) δ 30.4, 106.7, 111.3, 112.2, 114.7, 122.3,125.5, 126.0, 126.4, 130.6, 147.4, 148.3, 150.5, 155.2.

2-(p-Methylaminophenyl)-7-(2-tosylethoxy)imidazo[2,1-b]benzothiazole **(7)**

6 (150 mg, 0.51 mmol) was deprotonated with K_2CO_3 (138 mg, 2 eq.) in 100 mL DMF followed by drop wise adding ethylene glycol ditosylate (185 mg, 0.5 mmol) dissolved in 20 mL DMF at 80°C. The reaction mixture was cooled to room temperature after 30 min, 200 mL cold water added and extracted with 3 × 100 ml ethyl acetate. The organic phases were combined and washed with saturated sodium bicarbonate, brine, dried over sodium sulphate and concentrated. The crude product was purified by flash chromatography on a C18-RP cartridge. Compound **7** (119 mg, 48% overall yield, purity > 98 %) was confirmed by ESI-MS, [M+1]= 494.1; ^{1}H NMR (DMSO-d6) δ 2.40(3H, s), 2.72 (3H, s), 4.24 (2H, t), 4.38 (2H, t), 5.32, (1H, s), 6.58 (2H, d), 7.10 (1H, dd), 7.60 (6H, m), 7.80 (2H, d), 8.91 (1H, s); ^{13}C NMR (DMSO-d6) δ 27.3, 30.4, 67.3, 69.7, 107.1, 111.1, 112.5, 114.6, 126.5, 128.6, 130.0, 130.6, 131.3, 137.9, 144.5, 149.6.

2-(p-Methylaminophenyl)-7-(2-fluoroethoxy)imidazo[2,1-b]benzothiazole **(8)**

6 (10 mg, 0.034 mmol) was deprotonated with NaH (2 mg, ~2 eq.) in 2 ml DMF followed by reaction with 1 molar equivalent of 1-bromo-2-fluoroethane at 80°C. The reaction was followed by TLC and after 15 min no starting material was detected. The reaction mixture was cooled to room temperature, 100 ml water added, and the mixture extracted with 3 × 50 ml ethyl acetate. The combined organic

phase was washed with saturated sodium bicarbonate, brine, dried over sodium sulphate and concentrated. Compound **8** (9 mg, 78% overall yield, purity > 98 %) was confirmed by ESI-MS, [M+1]= 342.1; ^{1}H NMR (DMSO-d6) δ 2.71 (3H, s), 4.32 (2H, J_{HF} = 32Hz, m), 4.79 (2H, J_{HF} = 48Hz, m), 5.75 (1H, s), 6.59 (2H, d), 7.18 (1H, dd), 7.58 (2H, d), 7.68 (1H, d), 7.85 (1H, d), 8.40 (1H, s); ^{13}C NMR (DMSO-d6) δ 30.6, 68.6, 83.8, 100.6, 107.2, 111.2, 112.6, 115.5, 115.1, 126.5, 129.6, 131.1, 139.9, 150.1, 154.3, 156.3.

Procedures for radiosyntheses

One-step radiosynthesis of [^{18}F]2-(p-Methylaminophenyl)-7-(2-fluoroethoxy)imidazo[2,1-b]benzothiazole ([^{18}F]8)

[^{18}F]fluoride was produced through the ^{18}O(p,n)^{18}F nuclear reaction. The [^{18}F]fluoride was obtained in a 34 mM solution of K_2CO_3 (0.3 mL) and added to a 2 mL conical vial containing 0.5 mL dry MeCN and 15 mg (39.9 µmol) Kryptofix 2.2.2. The solvent was evaporated by heating at reduced pressure. Azeotropic drying was repeated three times with 0.5 mL portions of MeCN. **7** (2.4 mg, 5 µmol) was dissolved in 50 µl DMF and 250 µl MeCN and adding the solution to the dried kryptate ([K^+/2.2.2]$^{18}F^-$), the vial was then sealed and heated at 120 °C for 20 min. Thereafter the reaction mixture was diluted with 1 mL of MeCN: 0.1 % ammonium formate (40:60, $^{V}/_{V}$), loaded into a 2 mL injection loop and transferred onto a Chromolith® C18 (ID: 10 mm; length: 100 mm; Merck). The column was eluted with a mobile phase consisting of MeCN: 0.1 % ammonium formate (40:60, $^{V}/_{V}$) at a flow rate of 10 mL / min. In-line HPLC detectors included a UV detector (Sykam) set at 254 nm and a radioactivity detector (Bioscan Flow-Count fitted with a PIN detector). For animal experiments, the fraction containing the product was collected and diluted 1:1 with water. The mixture was applied to a Sep-Pak C18 Classic (Waters) and the cartridge subsequently washed with 10 mL water. The product was eluted with ethanol and diluted to the desired concentration with phosphate buffered saline. The pH of the final solution was between 7 and 8. Radiochemical and chemical purities were > 95 % as determined by analytical HPLC.

[^{18}F]**8** was prepared in a specific activity of > 3000 mCi/µmol (111 GBq/µmol) at end-of-synthesis.

Two-step radiosynthesis of [^{18}F]2-(p-Methylaminophenyl)-7-(2-fluoroethoxy)imidazo[2,1-b]benzothiazole ([^{18}F]**8**)

[^{18}F]fluoride was produced through the ^{18}O(p,n)^{18}F nuclear reaction. The [^{18}F]fluoride was obtained in a 34 mM solution of K_2CO_3 (0.3 mL) and added to a 2 mL conical vial containing 0.5 mL dry MeCN and 15 mg (39.9 µmol) Kryptofix 2.2.2. The solvent was evaporated by heating at reduced pressure. Azeotropic drying was repeated three times with 0.5 mL portions of MeCN. 2-[^{18}F]fluoroethyltosylate ([^{18}F]FEtTs) was prepared by dissolving 5 mg (12 µmol) of ethylene glycol-1,2-ditosylate in 250 µl MeCN and adding the solution to the dried kryptate [K^+/2.2.2]$^{18}F^-$), the vial was then sealed and heated at 90 °C for 5 min. The reaction vial was cooled to room temperature and 0.3 mL acetic acid 1% added to it. [^{18}F]FEtTs was purified by reversed phase HPLC (Lichrosorb C18 5µ; 10 mm × 250 mm CS-Chromatographie Service) eluted with MeOH : H_2O (50:50, volume/volume, flow rate 5 mL/min; k' = 5.7; preparative radiochemical yield 45 ± 5 %). After on-line fixation of the product on a Strata X cartridge (33 µm; 30 mg / 1 mL; Phenomenex) and drying of the product by argon-flush, the product was eluted with 0.15 mL DMF into the reaction vial. **6** (2.4 mg, 5 µmol) was dissolved in 150 µl DMF treated by sodium hydride (NaH, 2 mg, 80 µmol) and allowed to react for 5 min at ambient temperature. The solution was separated from the remaining NaH, transferred to the reaction vial at room temperature. The reaction vial was warmed to 90 ºC and kept at this temperature for 5 min. Thereafter the reaction mixture was diluted with 1 mL of MeCN:0.1 % ammonium formate (40:60, V/V), loaded into a 2 mL injection loop and transferred onto a Chromolith® C18 (ID: 10 mm; length: 100 mm; Merck). The column was eluted with a mobile phase consisting of MeCN : 0.1 % ammonium formate (40:60, V/V) at a flow rate of 10 mL / min. In-line HPLC detectors included a UV detector (Sykam) set at 254 nm and a radioactivity detector (Bioscan Flow-Count fitted with a PIN detector). For animal experiments, the fraction containing the product was collected and diluted 1:1 with water. The mixture was applied to a Sep-Pak C18 and the cartridge subsequently washed with 10 mL water. The product

was eluted with ethanol and diluted to the desired concentration with phosphate buffered saline. The pH of the final solution was between 7 and 8. Radiochemical and chemical purities were > 95 % as determined by analytical HPLC. [^{18}F]**8** prepared in a specific activity of > 3000 mCi/μmol (111 GBq/μmol).

Procedures for measurement of log $P_{Oct/PBS}$

Apparent drug lipophilicity was determined by a conventional partition method between 1-octanol and phosphate buffered saline (PBS), pH 7.4. The 1-octanol was saturated with PBS before use. Briefly, the no-carrier-added [^{18}F]**8**, contained in 0.2 mL PBS, was added 0.2 mL of 1-octanol in a 1.5 mL polypropylene Eppendorf vial. The vial was sealed and vigorously shaken at room temperature for 5 min. The mixture was then centrifuged at 3000·*g* for 10 min. A 100 μL aliquot from each of the two phases was drawn and their radioactivity content was determined in a NaI (Tl) well-type detector. The log $P_{oct/PBS}$ was calculated as follows:

log $P_{oct/PBS}$ = log (radioactivity concentration in the 1-octanol phase / radioactivity concentration in the PBS phase). The reported values represent the mean of three independent measurements.

General procedures for metabolite analyses

Male Balb-C mice were injected with 21-32 MBq of the compound of interest. At 10 and 30 min *p.i.* the animals were sacrificed and the tissue of interest was dissected.

The procedure used for the analysis of brain radioactivity was as follows: brain tissue homogenates were prepared immediately after dissection by mechanical homogenization of nitrogen-frozen samples followed by addition of 1 mL of phosphate buffered saline. The mixture was vigorously vortexed, and 1 mL of MeCN was added. After centrifugation for 5 min at 6.000•*g*, the supernatant was collected. Approximately 0.1 mL of the supernatant solution was analyzed using radio-HPLC [Nucleosphere 100,

5 µm; 10 × 150 mm (CS-Chromatographie); MeCN / 0.1 M ammonium formate 60:40, $^{V}/_{V}$ %. The outlet of the column was connected in-line with a solid-phase scintillation counter.

The amount of intact tracer (Ti) was calculated as follows:

$$Ti = [FT / FT + FM] \times EE \times ER \times 1 \cdot 10^{-2}$$

where FT [%] represents the amount of intact tracer and FM [%] the amount of metabolites as determined by radio-HPLC, corrected for extraction efficiency EE [%] from the plasma samples and the recovery ER [%] of activity from the HPLC. The extraction efficiency was in the range 64-95 % among the compounds investigated.

Animal experiments

The experiments were carried out with the approval of the institutional animal care committee (Regierung von Oberbayern, München, Germany), and in accordance with the German Animal Welfare Act (Deutsches Tierschutzgesetz). Animal husbandry followed the regulations of European Union (EU) guideline No. 86/609. BALB-C mice were obtained from Charles River laboratories Sulzfeld, Germany.

All imaging experiments were performed with homozygous APP/PS1 mice (B6;CB-Tg(Thy1-PSEN1*M146V/Thy1-APP*swe)-10Arte) (Artemis Pharmaceuticals, Cologne, Germany) on a congenic C57BL/6J genetic background. This mouse model is named ARTE10. The model has been extensively characterized regarding onset, progression, distribution and extent of Aβ plaque deposition as well as behavioral features.[2]

The animals were kept in a temperature-controlled environment (18-20 °C, 50-60 % relative humidity) on a 12:12 light/dark cycle with free access to a standard diet (Altromin 1326 mouse pellets, Altromin, Lage, Germany) and potable water. Mice were housed in type III cages (Ehret, Emmendingen, Germany), group size of ≤ 5 individuals with dust-reduced wood shavings as bedding. Upon arrival, all animals underwent an acclimatization period of ≥10 days prior to experiments.

Anesthesia

Inhalation anesthesia with isoflurane was used and the eyes of the animals were protected with dexpanthenol eye ointment. Anesthesia was initiated 15 min ahead of experimental procedures by placing the animal in a cage ventilated with oxygen (3.5 L/min) containing 3 % isoflurane. During the experiments, anesthesia for the duration of the procedure was maintained by adjusting the isoflurane content (0.6 % to 2 %) to ensure a respiratory rate in the range 80-100/min., Body temperature was held at 37 °C by employing a temperature-controlled heating pad.

Injection of tracer

Access to a lateral tail vein was made by a 30 gauge needle which was connected via a polythene tubing (0.28 mm inner diameter) to a 1 mL syringe. Prior to injection of the tracer, the catheter filled with isotonic sodium chloride solution. The catheter was stabilized at the injection site with superglue.

General biodistribution studies in wild-type mice

Biodistribution studies were performed in male Balb-C mice (body weight of 19-25 g). The mice were injected in a lateral tail vein with 4-6 MBq of a high specific activity (> 111 GBq /μmol) [^{18}F]**8** and [^{11}C]**1a** contained in 0.1-0.15 mL of a solution of isotonic phosphate buffered saline. Groups of mice were sacrificed at 5-30 min p.i. The radioactivity of weighed tissue samples was measured in a γ-counter. Data are expressed as percent of the injected dose per gram tissue (% I.D./g; mean ± sd, n = 5).

MRI Imaging

A brain MRI scan was performed on all tg and control mice within 0 to 6 days of μPET scan. Throughout MRI scan animals were under continuous 1.0% to 1.8% isoflurane anaesthesia with 2L/min oxygen flow. Eyes were protected with dexpanthenol ointment. Throughout MRI scan hypothermia was prevented with a heat storing gel pack (COLDHOT, 3M) preheated in a microwave oven.

We acquired T1 weighted brain images using a 3D turbo gradient echo (3D-TFE) sequence with an inversion pre-pulse (TR: 12ms, TE: 3.9ms, TI: 800ms, TFE Factor: 120, flip angle: 8°, NSA: 12,

acquired matrix MxP: 248x120, partitions: 60, FOV: 64x32x16mm, resolution: 0.26x0.27x0.26mm, reconstructed resolution: 0.13x0.13x0.13mm) on a Philips Achieva 1.5 T clinical MRI system with a 23 mm microscopy coil. Acquisition time was 46 minutes 11 seconds.

PET Imaging

All small-animal PET data were acquired with a Siemens Inveon PET/CT system (Siemens Medical Solutions, Knoxville, USA). After induction of anesthesia and accessing the tail vein, the animal was placed with its head located at the center of the field of view (12.7 × 10.0 cm ; axial × transaxial) and fixed in the CT module in prone head-first (HFP) position.

All animals underwent CT scan, first, and were automatically driven to the desired position for collecting PET data. In this manner, the predefined transformation matrix of the system was subsequently applied without the need of a manual co-registration process.

The radioactivity in the syringe was measured immediately before and after injection with a dose calibrator Capintec CRC® 15R (Capintec Inc, 6 Arrow Road Ramsey, NJ 07446) dose calibrator.

Dynamic data acquisition was performed in 3D-listmode for 45 min starting immediately with injection of the tracer. The emission data were normalized and corrected for decay and dead time. The images were reconstructed by means of filtered back-projection (FBP), using a ramp filter with a cut-off at the Nyquist frequency into 45 frames (45 × 60s). The image volume consisted of 128 × 128 × 159 voxels, each of a size of 0.78 × 0.78 × 0.80 mm.

***Ex vivo* evaluation**

Mice were killed by decapitation at the desired time point after injection. In the multi-modal experiments, mice were killed immediately after the end of the scan (30 min p.i.), and the entire brain was isolated within 6 ± 1 min. The brain was cut along the median sagittal line. One half was used for determination of the regional brain uptake and was dissected into: 1) olfactory bulb including ventral olfactory regions towards the olfactory tubercle, 2) cerebellum, 3) telencephalon and 4) the remaining brain structures (diencephalon and mid brain).

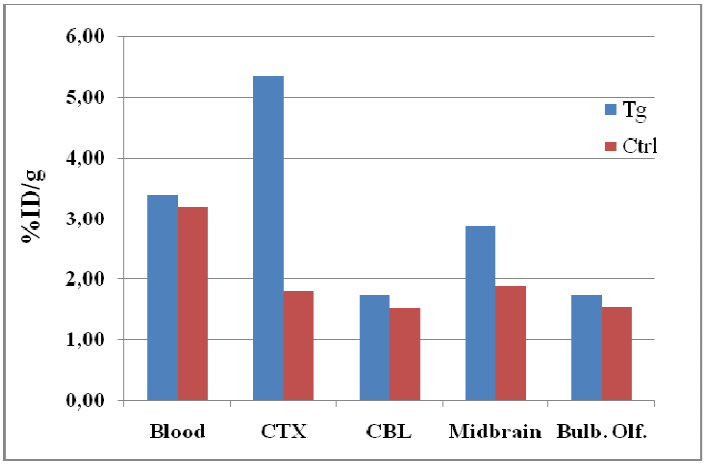


Chart 1. Regional biodistribution of [^{18}F]**8** 30 min p.i. in the Tg mouse

The radioactivity in weighed tissues was determined by means of an automatic NaI(Tl) well-type γ-detector (Wallac 1480-011 Automatic Gamma Counter, PerkinElmer, Waltham,MA, USA), related to a standard and used for calculation of the injected dose per gram tissue (% ID/g).

The other half of the brain was rapidly frozen for immediate sectioning on a cryostat. The frozen sections were mounted on dilute poly-L-lysine hydrobromide coated (MW > 300.000, (1:50) 0.01 % $^{w}/_{v}$ in water) microscopy slides. CNS sections were dried in ambient air and covered with scintillation foil. Three to five half axial sections were acquired in one scan. An optical image was taken at the end of acquisition.

Digital autoradiography

Digital autoradiographic images with a field of view of 24 × 32 mm were taken with the M40 series of μ-Imager^{TM} (Biospace lab, Paris, France) using 10 × 10 cm scintillating foils of thickness 13 ± 1.5 μm (Applied Scintillation Technologies, Harlow, England). The resolution for tritium is 15 μm, for F-18 25 μm, and the detection threshold for tritium is 0.4 cpm/mm^2, for carbon-11 and fluorine-18 0.04 cpm/mm^2.

Instrument acquisition was controlled with μ-Acquisition software. The pre-processing, quantitation and data management was handled with β-Vision+ software (both by Biospace lab).

Data processing and co-registration

MRI template was generated using SPM8. MRI data of all mice were normalized and a mean image calculated. µPET datasets and MRI template were loaded into Pmod 3.1 software (Pmod Technologies, Zürich, Switzerland) and brought into orthogonal orientation according to the Paxinos mouse atlas.

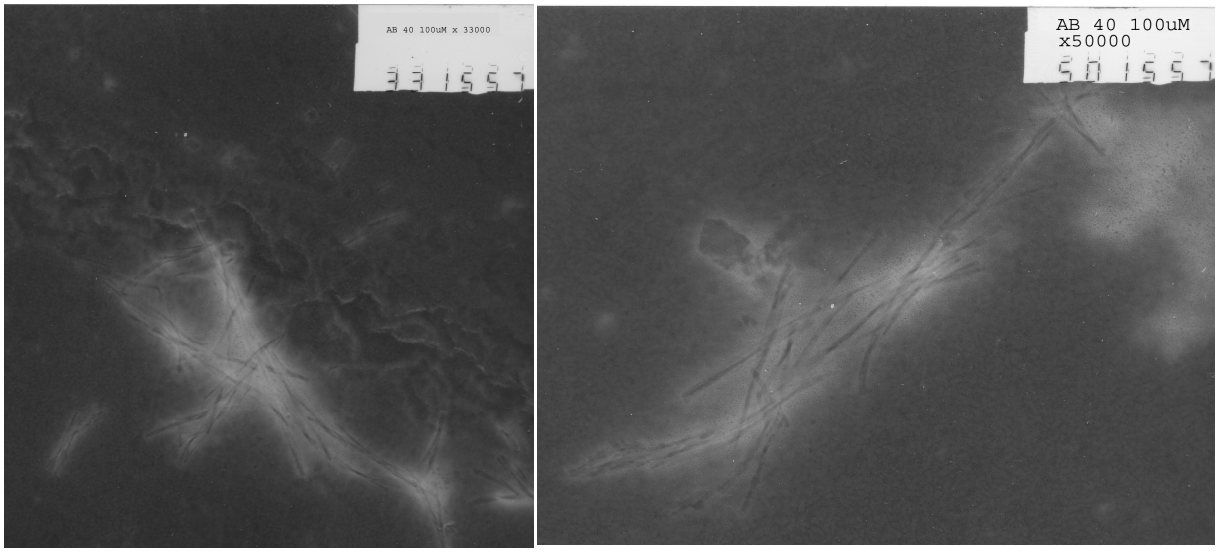
The MRI template was used for definition of volumes-of-interest (VOIs) in telencephalical and cerebellar regions. The time-activity curves represent the average VOI values over time. Pre-processed autoradiography data was finished with Adobe Photoshop CS4.

Determination of binding affinity of test compounds relative to [^{3}H]PiB

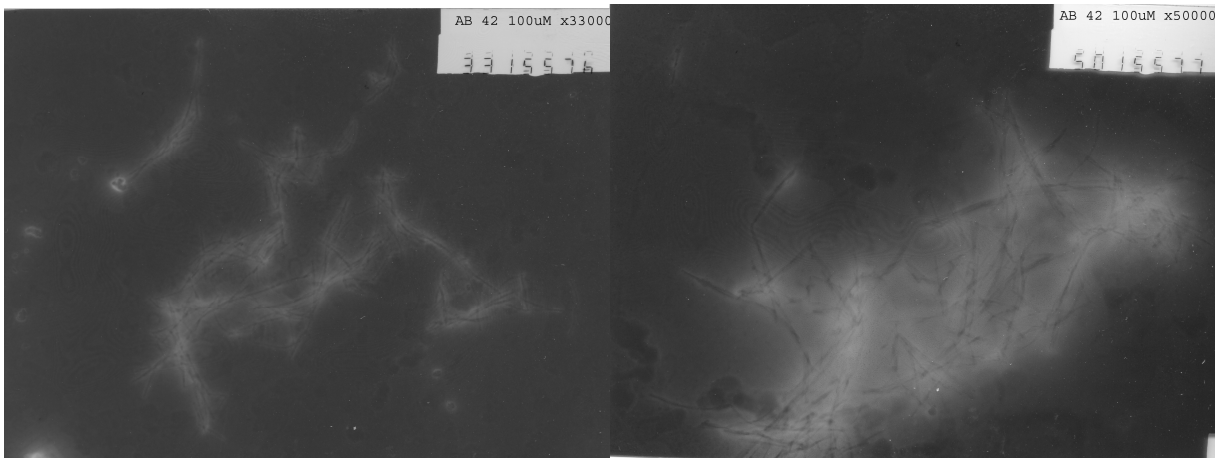
Preparation of A$\beta_{1\text{-}40}$ and A$\beta_{1\text{-}42}$ fibrils: Human A$\beta_{1\text{-}40}$ and A$\beta_{1\text{-}42}$ peptides (Bachem, Weil am Rhein, Germany) were incubated at 0.5 mg/mL in a solution consisting of 10 mM Na_2HPO_4, 1 mM EDTA (pH 7.4) at 37 °C for 48 h.[3] The formation of fibrils was confirmed by microscopy and binding of [^{3}H]PiB. Fibrils were either used immediately or aliquoted and subsequently stored at -80 °C until use.

Linearity of [^{3}H]**1a** (specific activity 3.15TBq/mmol, Quotient Bioresearch, Fordham, UK) binding was confirmed using the Aβ fibrils for a range of tracer (0.2 nM to 80 nM) and target (10 to 100µg/ml) concentrations and tracer incubation times (1h to 3h). The fixed concentration of 10 µg/mL of Aβ fibrils was titrated against 4.2 nM of [^{3}H]**1a** with twelve concentrations of testing compound (**8** or **1a in** 0.2nM to 1000nM concentration) dissolved in DMSO (The maximum final concentration of DMSO in the assays was 1%.) for 3h at room temperature (25°C) on a flat shaker at 240rpm (IKA-Werke, Staufen, Germany). Nonspecific binding was determined in the presence of 3µM unlabeled **1a** (PiB, ABX, Radeberg, Germany) including 1 h preincubation. The bound and free fractions were separated by vacuum filtration through GF/B glass filters (Whatman, Maidstone, UK) using a PerkinElmer harvester (PerkinElmer, 96 Micro B Filtermat) followed by 6 x 0.2 mL washes with ice cold 10 % EtOH in 10 mM Na_2HPO_4, pH 7.4. Filters containing the bound ligand were counted with a liquid scintillation counter (Wallac Trilux, 1450 Microbeta). Free tracer (octuples of all twelve [^{3}H]PiB concentrations) and background were measured with every experiment. Data were analyzed using GraphPad Prism

(GraphPad Software, San Diego, California, USA) to estimate the IC_{50} and Ki.

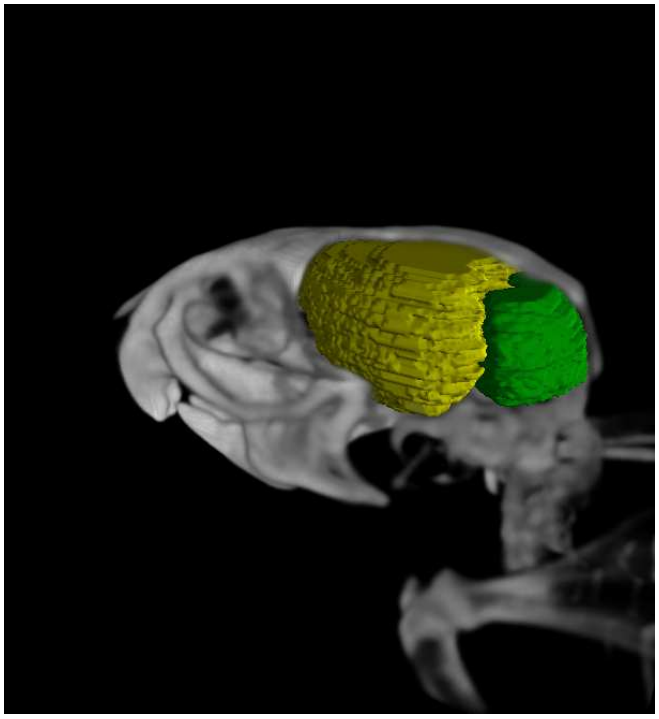


A **B**



C **D**

Supplementary Fig. 1 Transmission electron microscopy (TEM) images of Aβ aggregates fixed using a negative staining material, uranyl acetate. A and B are TEM images of $A\beta_{1\text{-}40}$ of 33000 and 50000 times magnification, respectively; C and D are TEM images of $A\beta_{1\text{-}42}$ of 33000 and 50000 times magnification, respectively.

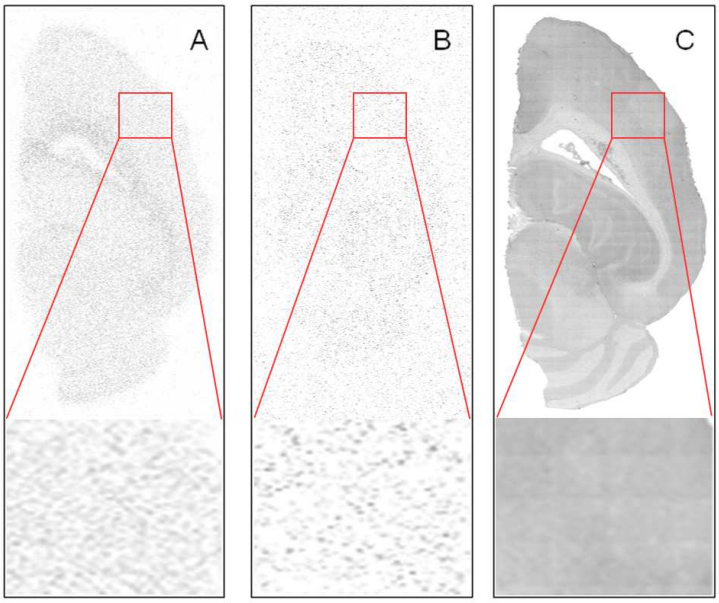


Supplementary Fig. 2 3D perspective of cortical (yellow) and cerebellar (green) VOI definition in µCT image of an APP/PS1 mouse (The left lateral posterior skull octant was cut out to show VOI definition).

Aβ plaque Immunohistochemistry

Frozen tissue sections from tg and control mouse brains were prepared by immersion in 4% formaldehyde (Carl Roth) for 30min followed by 70% formic acid (Merck) for 15 min at RT for epitope retrieval. Sections were then blocked with 3% bovine serum albumin (BSA, Fluka/Sigma) in PBS for 15 min and simultaneously probed with the Rabbit anti-Aβ (x-40) AHP676 (AbD Serotec), and mouse anti-Aβ (x-42) G2-13 (The Genetics Company) were used as primary antibodies. Cy5-Fluorophor-coupled donkey-anti-mouse (Jackson ImmunoResearch) and Alexa488-Fluorophor-coupled donkey-anti-rabbit (Molecular Probes) were used as secondary antibodies. For immunofluorescence staining, sections were pretreated with 4% formaldehyde (Carl Roth) for 30 min followed by 70% formic acid (Merck) for 15 min at RT for epitope retrieval. Sections were then blocked with 3% bovine serum albumin (BSA, Fluka/Sigma) in PBS for 15 min and simultaneously probed with the two primary antibodies (G2-13: 1:5000, AHP676: 1:50) diluted in 1% BSA/PBS over night at 4 °C, washed with PBS, blocked again

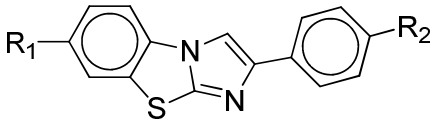
with 3% BSA/PBS and simultaneously incubated with Alexa488 and Cy5 fluorophore-conjugated secondary antibodies, washed, and cover-slip mounted with ProLong Gold Antifade mounting medium (Invitrogen/Molecular Probes). Finally, the slides were analyzed under Zeiss AxioImager Z1 microscope with Zeiss AxioCam MRm Rev 3, Zeiss AxioVision 4.8.1.0 Software, Zeiss 38 HE (GFP) for FITC/Alexa488 and Zeiss 50 (Cy5) for Cy5 filters, EC Plan-Neofluar 20x/0.50 M27 objective, and 1388x1040 pixels (0.32 micrometer/pixel) resolution.



Supplementary Fig. 3. *Ex vivo* dual-tracer autoradiography of a 12 µm thick axial section of control mouse brain killed 45 minutes after co-injection of [^{18}F]**8** and [^{3}H]**1a** superimposed to the optical image. (A) Separated image of [^{18}F]**8** autoradiography; (B) Separated image of [^{3}H]**1a** autoradiography; (C) fused IHC images of Anti- $A\beta_{40}$ and Anti- $A\beta_{42}$ (gray scale).

Description of the HPLC systems including k′-values

Supplementary Table 1. Experimental HPLC[a] data for confirming purity of compounds 3-8.



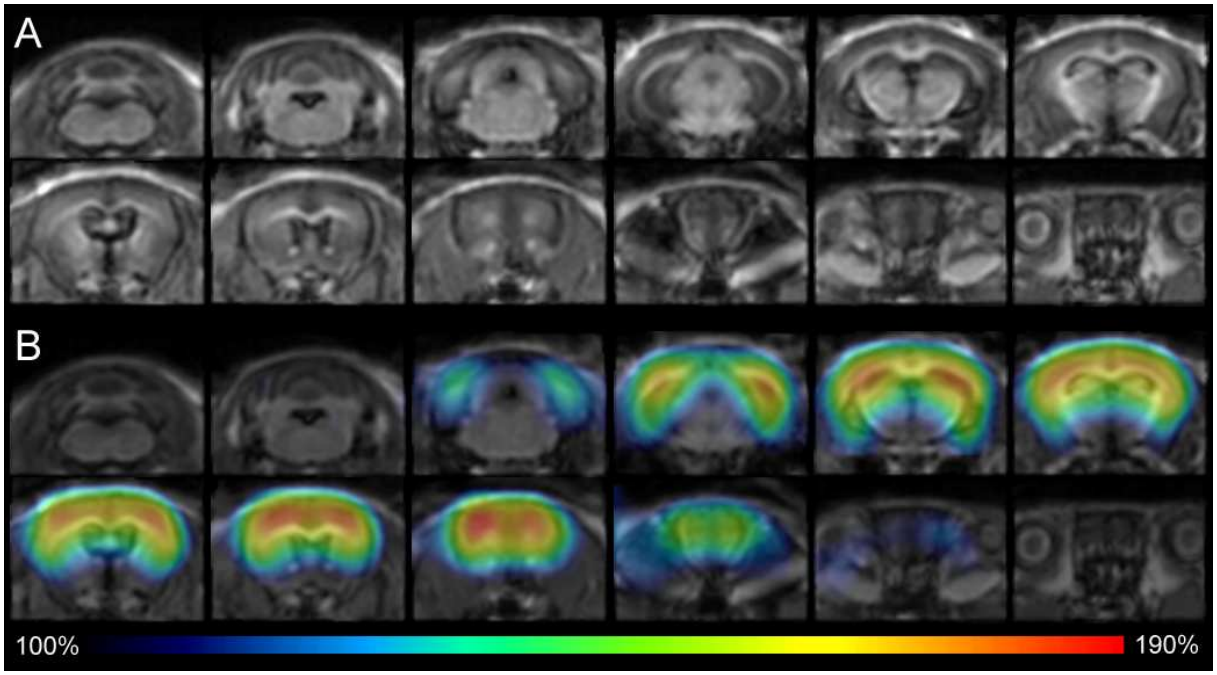
R_1	R_2	Compound	HPLC 1[b] k′[d], purity (%)	HPLC 2[c] k′[d], purity (%)
CH_3O-	-NH(COMe)	3	3.1, 98.7	8.3, 99.1
CH_3O-	-NH_2	4	1.5, 98.8	2.8, 99.0
CH_3O-	-NHMe	5	3.0, 99.0	8.4, 98.7
HO-	-NHMe	6	1.8, 98.8	5.1, 98.9
$TsO(CH_2)_2$-	-NHMe	7	8.6, 98.3	14.8, 98.6
$F(CH_2)_2$-	-NHMe	8	5.1, 99.2	9.2, 99.4

[a] The in line UV detector was a Sykam Model S3210 (Sykam, Fürstenfeldbruck, Germany) set at 254 nm.

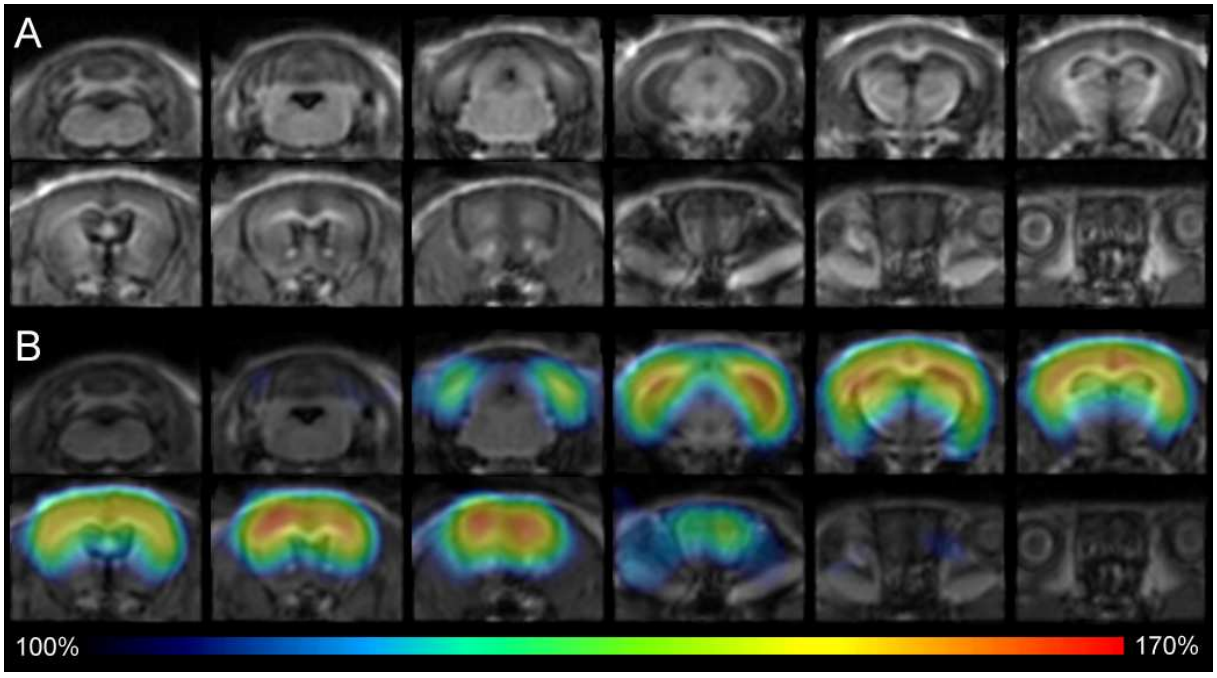
[b] HPLC system 1 was a Chromolith® reverse phase column (4.6 × 100 mm, Merck KGaA, Darmstadt, Germany) eluted with acetonitrile / 0.1 M ammonium formate (50:50 % $^{v}/_{v}$) mobile phase mixture. The flow rate was 5 mL / min.

[c] HPLC system 2 was Chromolith® reverse phase column (4.6 × 100 mm) eluted with acetonitrile / 0.1 M ammonium formate (37.5: 62.5 % $^{v}/_{v}$). The flow rate was 5 mL / min.

[d] The capacity factor, *k*', is calculated as follows: $k'=(t_R-t_0) / t_0$ where t_R = retention time of the substance in question (min) and t_0 = dead volume of the column (mL) / flow (mL × min^{-1}).



Supplementary Fig. 4 Summed images of [^{18}F]**8** (36 to 45 minutes) post injection. Coronal view of (A) MRI template from cerebellum to bulbus olfactorius and (B) MRI template overlaid with smoothed (3D Gaussian 1.0 mm) parametric PET image representing cerebellum-normalized mean signal of APP/PS1 tg mice in percent of cerebellum-normalized mean signal of controls. The scale bar indicates percent uptake in the region of interest relative to that in the cerebellum.



Supplementary Fig. 5 Summed images of [^{18}F]**8** (26 to 30 minutes post injection). Coronal view of (A) MRI template from cerebellum to bulbus olfactorius and (B) MRI template overlaid with smoothed (3D Gaussian 1.0mm) parametric PET image representing cerebellum-normalized mean signal of APP/PS1 tg mice in percent of cerebellum-normalized mean signal of controls. The scale bar indicates percent uptake in the region of interest relative to that in the cerebellum.

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