SUPPORTING INFORMATION

Development of an Improved Radioiodinated 2-Phenylimidazo[1,2-a]Pyridine for Non-Invasive Imaging of Amyloid Plaques

Behrooz Hooshyar Yousefi,^{a,b} André Manook,^a Boris von Reutern,^a Markus Schwaiger,^a Alexander Drzezga,^a Hans-Jürgen Wester^{b,a} and Gjermund Henriksen^a

 ^a Klinikum rechts der Isar, Technische Universität München, Department of Nuclear Medicine, Ismaninger Straße 22, 81675 Munich, Germany.
^b Lehrstuhl für Pharmazeutische Radiochemie, Walther-Meissner-Straße 3, 85748 Garching, Germany.

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Experimental Section

General methods

All commercial reagents and solvents were used without further purification unless otherwise specified. Microwave reactions were performed in dedicated 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.

The purity of all compounds were measured (>95%) on two different HPLC-systems: System 1 was a Chromolith[®] RP18 4.6 × 100 mm reverse phase column (VWR) eluted with acetonitrile / 0.1 M ammonium formate (50:50, v/v) mobile phase mixture at flow rate of 5.0 mL / min (HPLC 1). 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. (HPLC 2). Both chromatography systems were fitted with a UV detector (Sykam Model S3210 set at 254 nm; Sykam, Fuerstenfeldbruck, Germany.

General procedure

The 2-aminopyridine (1 mmol) and the phenacylhalide (1.1 equivalent) were dissolved or suspended in EtOH (5 mL) and refluxed for 1–16 hours. 2-Phenyl-imidazo[2,1-*a*]pyridines which precipitated were collected from reaction mixture after cooling down to room temperature by filtering, washed with Diethylether (2 × 2mL) and finally dried, the yields are in the range 38–85% as shown in Scheme 1-2 (Table 1 for a full list of compounds). All the products and intermediates were characterized by mass spectrometry, NMR and their purities checked by HPLC.

2-(4-dimethylaminophenyl)-6-iodoimidazo[1,2-a]pyridine (4a)

The general synthetic procedure was used and **4a** with 62.9% yield obtained, m.p. 232 °C, [M+1] 364.1, ¹H-NMR (500 MHz, DMSO-d6) δ 8.22 (m, 1H), 7.81 (d, 2H), 7.69 (s, 1H), 7.48 (d, 1H), 7.18 (dd, 1H), 6.82 (d, 2H), 3.08 (s, 6H).

2-(4-bromophenyl)-6-iodoimidazo[1,2-a]pyridine (4b)

The general synthetic procedure was used and **4b** with 56.2% yield prepared, [M+1] 398.9, 400.9, ¹H NMR (500 MHz, DMSO-d6) δ 9.01 (s, 1H), 8.44 (d, *J* = 5.8 Hz, 1H), 7.90 (m, 2H), 7.68 (m, 2H), 7.58 (dd, *J* = 55.4, 9.5 Hz, 2H); ¹³C NMR (126 MHz, DMSO) δ 144.2, 143.4, 142.7, 135.7, 135.4, 132.5, 132.9, 131.0, 121.8, 117.6, 117.4, 110.3, 78.5.

6-iodo-2-(4-methoxyphenyl)imidazo[1,2-a]pyridine (4c)

The general synthetic procedure was used and **4c** with 76.5% yield prepared, [M+1] 351.0, ¹H NMR (500 MHz, DMSO-d6) δ 9.11 (s, 1H), 8.43 (s, 1H), 7.88 (m, 3H), 7.62 (d, *J* = 9.4 Hz, 1H), 7.11 (td, *J* = 5.4, 2.2 Hz, 3H), 3.83 (d, *J* = 1.3 Hz, 4H).¹³C NMR (126 MHz, DMSO-d6) δ 161.1, 156.2, 141.4, 137.8, 134.0, 133.3, 128.2, 122.2, 116.2, 110.4, 109.8, 80.3, 56.1.

2-(3-bromo-4-dimethylaminophenyl)-6-iodoimidazo[1,2-a]pyridine (4d)

The general synthetic procedure was used and the **4d** with 46.2% yield obtained, [M+1] 441.9, 443.9, ¹H NMR (500 MHz, DMSO-d6) δ 8.51 (dd, *J* = 1.7, 0.9 Hz, 1H), 7.94 (m, 1H), 7.75 (d, *J* = 8.8 Hz, 1H), 7.53 (dd, *J* = 9.3, 1.7 Hz, 1H), 7.46 (dd, *J* = 9.3, 0.9 Hz, 1H), 7.36 (d, *J* = 1.4 Hz, 1H), 6.84 (m, 1H), 6.77 (d, *J* = 8.9 Hz, 1H), 2.97 (s, 6H); ¹³C NMR (126 MHz, DMSO) δ 150.6, 143.8, 133.8, 130.0, 129.3, 128.9, 127.4, 118.6, 113.0, 112.4, 107.8, 75.9, 40.9.

6-bromo-2-(4-methoxyphenyl)imidazo[1,2-a]pyridine (4e)

The general synthetic procedure was used and the **4e** with 84.1% yield prepared, [M+1] 303.0, 305.0 ¹H NMR (500 MHz, DMSO-d6) δ 9.12 (dd, *J* = 1.9, 0.9 Hz, 1H), 8.49 (s, 1H), 7.90 (m, 2H), 7.79 (m, 2H), 7.12 (m, 2H), 3.83 (s, 3H); ¹³C NMR (126 MHz, DMSO) δ 160.9, 140.8, 133.86, 129.14, 128.8, 128.1, 121.1, 116.0, 115.6, 115.3, 110.4, 109.6, 56.3.

6-chloro-2-(4-methoxyphenyl)imidazo[1,2-a]pyridine (4f)

The general synthetic procedure was used and the **4f** with 56.2% yield prepared, [M+1] 259.1, 498 mg, yield 96.5 %, HPLC > 99 %. MS 259.7 [M+H], ¹H NMR (500 MHz, DMSO-d6) δ 9.15 (t, *J* = 1.3 Hz, 1H), 8.58 (s, 1H), 7.91 (m, 4H), 7.15 (m, 2H), 3.85 (s, 3H); ¹³C NMR (126 MHz, DMSO) δ 161.4,

140.7, 138.2, 133.9, 133.2, 131.2, 128.8, 127.6, 120.2, 115.3, 114.4, 111.0, 110.7, 56.6.

2-(3,4-difluorophenyl)-6-iodoimidazo[1,2-a]pyridine (4g)

The general synthetic procedure was used and **4g** with 38.2% yield prepared, [M+1] 357.0, ¹H NMR (500 MHz, DMSO-d6) δ 9.17 (m, 1H), 8.58 (s, 1H), 8.07 (ddd, J = 11.8, 7.6, 2.2 Hz, 1H), 7.90 (dd, J = 9.3, 1.7 Hz, 1H), 7.82 (m, 1H), 7.66 (m, 2H); ¹³C NMR (126 MHz, DMSO) δ 151.6, 149.7, 141.6, 138.9, 137.8, 133.4, 124.1, 123.4, 119.6, 117.0, 116.7, 111.3, 80.6.

2-(2-bromo-3-methoxyphenyl)-6-iodoimidazo[1,2-a]pyridine (4h)

440 mg 2-amino-5-iodopyridine (2.0 mmol; 220,01 g/mol) and 678 mg 2-bromo-1-(2-bromo-3methoxyphenyl)ethanone (2.2 mmol; 307.97 g/mol) in 5 ml ethanol were stirred at reflux condition for 72h. The product as white precipitate after cooling down of reaction mixture was filtered and with Diethylether (2 × 2mL) washed and finally dried (810 mg, 1.91 mmol; 429.05 g/mol, 80% yield). HPLC analysis of product showed purity of 96,2 %, MS 429.0 ($[M+H]^+$), 430.9, (500 MHz, ¹H-NMR CDCl₃) δ 8.70 (s, *J*=1.5 Hz, 1H), 8.50 (d, *J*=9.4 Hz, 1H), 8.10 (d, *J*=9.4 Hz, 1H), 7.83 (s, *J*=2.4 Hz, 1H), 7.79 (d, *J*=8.0 Hz, 1H), 7.50 (t, *J*=8.0 Hz, 1H), 7.10 (d, *J*=8.5, 2.6 Hz, 1H), 4.0 (3H, s); ¹³C NMR (126 MHz,) δ 155.8, 143.9, 133.3, 132.2, 131.2, 130.8, 127.2, 118.3, 113.7, 111.5, 109.3, 76.7, 57.0.

2-(4-chloro-3-nitrophenyl)-6-iodoimidazo[1,2-a]pyridine (4i)

The general synthetic procedure was used and **4i** with 56.8% yield prepared, [M+1] 400.0, ¹H NMR (500 MHz, DMSO-d6) δ 9.09 (t, J = 1.2 Hz, 1H), 8.63 (m, 2H), 8.25 (dd, J = 8.4, 2.1 Hz, 1H), 7.91 (d, J = 8.4 Hz, 1H), 7.73 (dd, J = 9.3, 1.7 Hz, 1H), 7.59 (d, J = 9.3 Hz, 1H); ¹³C NMR (126 MHz, DMSO) δ 156.1, 148.9, 143.0, 139.3, 137.0, 133.3, 131.6, 123.8, 123.5, 117.3, 112.0, 79.4.

2-(4-chloro-3-nitrophenyl)-6-chloroimidazo[1,2-a]pyridine (4j)

The general synthetic procedure was used and the **4j** with 56.2% yield prepared, [M+1] 308.0, ¹H NMR (500 MHz, DMSO-d6) δ 9.19 (t, J = 1.2 Hz, 1H), 8.69 (m, 2H), 8.27 (dd, J = 8.4, 2.1 Hz, 1H), 7.93 (d, J = 8.4 Hz, 1H), 7.71 (dd, J = 9.3, 1.7 Hz, 1H), 7.56 (d, J = 9.3 Hz, 1H); ¹³C NMR (126 MHz, DMSO) δ 148.7, 145.1, 135.2, 132.3, 130.6, 127.8, 123.4, 119.3, 112.6.

2-(3,4-difluorophenyl)-6-bromoimidazo[1,2-a]pyridine (4k)

The general synthetic procedure was used, **4k** with 56.2% yield prepared, [M+1] 309.0, 311.0, ¹H NMR (500 MHz, DMSO-d6) δ 9.12 (m, 1H), 8.58 (s, 1H), 8.07 (ddd, J = 11.8, 7.6, 2.2 Hz, 1H), 7.90 (dd, J = 9.3, 1.7 Hz, 1H), 7.82 (m, 1H), 7.66 (m, 2H); ¹³C NMR (126 MHz, DMSO) δ 151.6, 149.7, 141.6, 138.9, 137.8, 133.4, 124.1, 123.4, 119.6, 117.0, 116.7, 111.3, 80.6.

2-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-6-bromoimidazo[1,2-a]pyridine (4l)

The general synthetic procedure was used, **4I** with 56.2% yield was prepared, [M+1] 331.0, 333.0, ¹H NMR (500 MHz, DMSO-d6) δ 8.71 (s, 1H), 8.24 (s, 1H), 7.66 (m, 2H), 7.42 (m, 1H), 7.36 (dd, J = 9.3, 1.7 Hz, 1H), 6.96 (m, 1H), 4.34 (s, 4H).

2-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-6-chloroimidazo[1,2-a]pyridine (4m)

The general synthetic procedure was used and the **4m** with 56.2% yield prepared, [M+1] 287.1, ¹H NMR (500 MHz, DMSO-d6) δ 8.79 (s, 1H), 8.29 (s, 1H), 7.63 (m, 2H), 7.47 (m, 1H), 7.38 (dd, J = 9.3, 1.7 Hz, 1H), 6.93 (m, 1H), 4.31 (s, 4H).

2-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-6-iodoimidazo[1,2-a]pyridine (4n)

The general synthetic procedure was used, **4n** was prepared with 56.2% yield, ESI-MS [M+1] 379.0, ¹H NMR (500 MHz, DMSO-d6) δ 8.89 (s, 1H), 8.29 (m, 2H), 7.43 (m, 3H), 6.91 (d, *J* = 9.3 Hz, 1H), 4.23 (s, 4H).

2-(4-dimethylaminophenyl)-6-(trimethylstannyl)imidazo[1,2-a]pyridine (6)

Similar synthetic procedure to **5** was used and trimethylstannyl derivative **6** starting from **4a** with 48% yield obtained, ESI-MS 402.1, 400.1, 398.1, ¹H-NMR (CDCl₃): 8.43 (t, J = 1.1, 1H); 8.21 (d, J = 8.6, 1H); 7.62 (m, 2H); 7.55 (dd, J = 8.5, 1.5 Hz, 1H) 7.26 (m, 1H); 7.01 (d, J = 8.6 Hz, 1H); 6.95 (d, J = 8.5, 1H); 3.06 (s, 6H), 0.36 (s, 9H). ¹³C-NMR (CDCl₃): 155.6, 154.3, 145.0, 142.9, 135.3, 130.5, 128.1, 122.6; 114.5; 113.4; 111.9, 42.3, 5.9. ESI-MS: 437.0 ([M+1]), 435.0, 439.0.

Procedures for radiosyntheses

$[^{125}I]_{2-(4-dimethylaminophenyl)-6-iodoimidazo[1,2-a]pyridine ([^{125}I]_{4a})$ and $[^{125}I]_{2-(4-bromophenyl)-6-iodoimidazo[1,2-a]pyridine ([^{125}I]_{4b})$

Hydrogen peroxide (50 µL, 3% w/v) was added to a mixture of 50 µL of the corresponding trimethyltin precursor (1 µg/µL EtOH), 50 µL of 1 N HCl, and [¹²⁵I]NaI (1-5 mCi) in a sealed vial. The reaction was allowed to proceed for 30 min at room temperature and terminated by addition of 100 µL of saturated NaHSO₃. To reaction mixture was added ethanol (100 µL) after neutralization with saturated sodium bicarbonate solution. The combined mixture was purified by HPLC using a reversed-phase, Chromolith[®] C18 (ID: 10 mm; length: 100 mm; Merck), with an isocratic solvent consisting of 70% acetonitrile: 30% ammonium formate (0.1 %) at a flow rate of 3.0 mL/min. For animal experiments, the no-carrier-added products, the fraction containing the product, was collected and diluted 1:1 with water. The mixture was applied to a Sep-Pak C18 classic 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. The final [¹²⁵I]**4a**/[¹²⁵I]**4b**, with a specific activity of >2000 Ci/mmol and >95% radiochemical purity, was stored at -20 °C up to 6 weeks for in vitro stability and autoradiography studies.

[¹²⁴I]2-(4-bromophenyl)-6-iodoimidazo[1,2-a]pyridine ([¹²⁴I]4b)

Similar labeling procedure to $[^{125}I]$ **4b** was used, $[^{124}I]$ **4b** with RCY of 25 ± 3 % and >98% radiochemical purity obtained.

Procedures for measurement of logP

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 [¹²⁵I]**4a** or [¹²⁵I]**4b**, 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 $3000 \cdot g$ for 10 min. A 100 µL aliquot from each of the two phases was drawn and their radioactivity content were determined in a NaI (Tl) well-type detector. The log $P_{\text{oct/PBS}}$ was calculated as follows:

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

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 experiments performed homozygous APP/PS1 mice ex vivo were with (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 A β plaque deposition as well as behavioral features.¹

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 \leq 5 individuals with dust-reduced wood shavings as bedding. Upon arrival, all animals underwent an acclimatization period of \geq 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 n.c.a. [¹²⁵I]**4a** and [¹²⁵I]**4b** contained in 0.1-0.15 ml of a solution of isotonic phosphate buffered saline. Groups of mice were sacrificed at 5, 30, 60, 120 min and 24h 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 ≥ 4).



Supplementary Fig. 1 Biodistribution results of [¹²⁵I]4a, IMPY.



Supplementary Fig. 2 Biodistribution results of [¹²⁵I]4b, Br-IMPY.

General procedures for metabolite analyses

Male Balb-C mice were prepared according to the procedure described for the biodistribution studies and were injected with 8-12 MBg of n.c.a. [¹²⁵I]**4b** in a total volume of 0.1 mL PBS. The animals were sacrificed after 5, 10, 30 and 60 min p.i. and blood and brain were collected. Blood was collected in heparinized tubes and centrifuged (5 min, 5.000 rpm) for plasma isolation. Brain rapidly rinsed and immersed in ice-cold 50 mM TRIS / 0.2 M sucrose buffer, pH 7.4. The organs were subsequently homogenized with a manual homogenizer for 5 min, the mixture vigorously vortexed, and 1 mL of MeCN added. After centrifugation for 5 min at 6.000•g, the supernatant was collected. followed by centrifugation (10 min, 12.000 rpm) and the supernatants were used for further evaluation. Samples $(300 \ \mu L)$ of plasma and homogenized brain were transferred to an ultrafiltration device (Vivacon 500, membrane: 30.000 MWCO HY, Sartoriusstedium biotech GmbH, Goetfingen, Germany) followed by centrifugation (10 min, 12.000 rpm) for the separation of proteins. Approximately 0.1 mL of the supernatant solutions (15000 cpm) as well as a sample of $[^{125}I]$ 4b (all sample were approximately 15000 cpm, measured by γ -counter) were applied on a preparative TLC glass plate (PTLC silica gel with F₂₅₄ indicator on glass plates with 4×20 cm preconcentration zone) for the quantification of the radiolabeled metabolic species and eluted with a mixture of 12% MeOH and 88% CH₂Cl₂. The PTLC was detected by Phospho-Imager (CR 35 BIO, Isotopenmeßgeräte GmbH Straubenhardt, Germany) and the resulting

images analyzed using AIDA Image Analyzer software.

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

$$Ti = [FT / FT + FM] \times EE \times ER \times 1.10^{-2}$$

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 58-92 % among the compounds investigated.

Ex vivo evaluation

Mice were killed by decapitation at the desired time point after injection 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).

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 (mol wt > 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 -ImagerTM (Biospace lab, Paris, France) using 10×10 cm scintillating foils of thickness $13 \pm 1.5 \mu$ m

(Applied Scintillation Technologies, Harlow, England). The resolution with tritium is 20 μ m, for carbon-11 it is ~40 μ m, the detection threshold for tritium is 0.4 cpm/mm², for carbon-11 and fluorine-18 it is 0.7 cpm/mm².

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).

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

Human A β_{1-40} and A β_{1-42} peptides (Bachem, Weil am Rhein, Germany) were incubated at 0.5 mg/ml in a solution consisting of 10 mM Na₂HPO₄, 1 mM EDTA (pH 7.4) at 37 °C for 48 h. The formation of fibrils was confirmed by microscopy and binding of [³H]**PiB**. Fibrils were either used immediately or aliquoted and subsequently stored at -80 °C until use.

Solutions of the test substances or **PiB** (ABX Biochemicals, Radeberg, Germany), all of > 98% purity according to analytical HPLC, and **PiB** were prepared as 1–10 mM dimethyl sulfoxide (DMSO) stock solutions before dilution into the assay buffer. The maximum final concentration of DMSO in the assays was 1%. All assays were performed in 10 % EtOH in 10 mM Na₂HPO₄, pH 7.4. The incubation was performed at 25 °C for 180 min. 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 Na₂HPO₄, pH 7.4. Filters containing the bound ligand were counted with a liquid scintillation counter (Wallac Trilux, 1450 Microbeta). The inhibition of [³H]**PiB** binding to fibrils of A β_{1-40} and A β_{1-42} was determined at 100 nM concentration of the test compound in question.

Linearity of $[{}^{3}H]$ **PiB** (specific activity 3.15TBq/mmol, Quotient Bioresearch, Fordham, UK) binding was confirmed using the A β 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 A β fibrils was titrated against 4.2 nM of $[{}^{3}H]$ **PiB** with twelve concentrations of testing compounds (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 3μ M unlabeled **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 × 0.2 mL washes with ice cold 10 % EtOH in 10 mM Na₂HPO₄, 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 [³H]PiB concentrations) and background were measured with every experiment. Data were analyzed using GraphPad Prism S14 (GraphPad Software, San Diego, California, USA) to estimate the IC50 and Ki.



Supplementary Fig. 3 Transmission electron microscopy (TEM) images of A β aggregates fixed using a negative staining material, uranyl acetate. A and B are TEM images of A $\beta_{1.40}$ of 33000 and 50000 times magnification, respectively; C and D are TEM images of A $\beta_{1.42}$ of 33000 and 50000 times magnification, respectively.

Aβ plaque Immunohistochemistry

Frozen tissue sections from tg and control mouse brains were prepared by immersion in 4% formaldehyde (Carl Roth) for 30 min followed by 70% formic acid (Merck) for 15 min at RT for epitope retreavel. Sections were then blocked with 3% bovine serum albumin (BSA, Fluka/Sigma) in PBS for 15 min and simultaneously probed with the Rabbit anti-A β (x-40) AHP676 (AbD Serotec), and mouse anti-Aß (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 Cv5 fluorophore-conjugated secondary antibodies, washed, and coverslip 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.

Thioflavin S staining

Fluorescent staining with Thioflavin S for frozen material was performed similar as reported before¹. Deep frozen mouse brain sections were dried in ambient air for 15 min, immersion-fixed in ice-cold 4 % (w/v) paraformaldehyde (Carl Roth, Karlsruhe, Germany) for 20 min. Sections were equilibrated in water twice for 2 min. Thioflavin S was dissolved at 1 % (w/v) in water, and the solution was filtered. Sections were immersed in 1% Thioflavin S for 30 min at RT and kept dark, rinsed twice for 2 min in water, and differentiated in two changes of 80 % ethanol (5 min and 1 min), washed in three changes of

water (2 min each) and mounted in ProLong Gold antifade mounting medium (Invitrogen, Karlsruhe, Germany) with cover slips. Thioflavin S staining of all sections for A β plaque quantification was performed in a single staining procedure to ensure best possible comparability.

Microscopy

Fluorescence microscopy for entire-view high-resolution MosaiX pictures of horizontal mouse brain sections were created with an AxioImager Z.1 microscope (Carl Zeiss Microimaging, Munich, Germany) on a Zeiss CAN-Bus motor stage (Merzhäuser, Germany) using a 20x/0.5 M27 EC Plan-Neofluar Zeiss lens and Zeiss filter sets no. 49 (DAPI), no. 38 (HE Green Fluorescent Protein), no. 43 (HE DsRed), no. 47 (HE Cyan Fluorescent Protein) and no. 50 (Cy5). Micrographs were acquired with an AxioCam MRm Rev. 3.0 (Carl Zeiss Microimaging, Munich, Germany) camera. Data acquisition was controlled with AxioVision 4.8.1 and conversion of very large tiled MosaiX images to single 16-bit grayscale TIFF files per channel was performed with AxioVision 4.8.2 SE64.

References

1. Willuweit, A.; Velden, J.; Godemann, R.; Manook, A.; Jetzek, F.; et al. Early-Onset and Robust Amyloid Pathology in a New Homozygous Mouse Model of Alzheimer's Disease. *PLoS ONE* **2009**, 4(11): e7931. doi:10.1371/journal.pone.0007931.