Electronic Supplementary Information

Radiosynthesis and characterization of astemizole derivatives as lead compounds toward PET imaging of τ-pathology

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General: Chemicals where obtained from Alfa-Aesar (Alfa Aesar UK Ltd, Heysham, Morecambe, UK), Fisher Scientific (Fisher Scientific UK Ltd, Loughborough, UK) and Sigma-Aldrich (Sigma-Aldrich Co. Ltd, Poole, UK). Solid phase extraction cartridges were obtained from Waters (Waters Ltd, Elstree, UK). Analytical HPLC was performed on an Agilent 1100 series HPLC system (Agilent Technologies UK Ltd, Wokingham, UK), consisting of a G1312 A gradient pump and a G1314 variable wavelength UV-detector. A Bioscan (Bioscan Inc., Washington DC, USA) 1" thallium doped sodium iodide NaI(Tl) detector with Flow-Count B-FC-4000 analogue/digital interface were used for radioactivity detection. Lablogic Laura 3 and Laura 4 software (Lablogic Systems Ltd, Sheffield, UK) were used for data acquisition and evaluation. For screening of reaction conditions and quality control, a Chromolith RP18e (5μm) 0.4 mm x 100 mm column (Merck KGaA, Darmstadt, Germany) at a flow rate of 2 mL/min (7 mM NH_4OH-Acetonitril gradient) or a Phenomenex Luna C18(2) (5 μm) 0.46 x 250 mm column at a flow rate of 1.5 mL/min (MeCN/0.1% aqueous Et_3N 60/40) were used as stationary phase. The semi-preparative radioHPLC system is equipped with a Sykam S1122 isocratic pump, a UV detector (K2001, Knauer) and a radiodetector (built-in TRACERlab FX F-N). The system is fitted with a reversed-phase HPLC column (Phenomenex Luna C18 10 x 250mm 5μm) eluted with a 50/50 (v/v) mixture of acetonitrile and 0.1% aqeous Et_3N at 3ml/min. The UV and radioactive traces were monitored with the TRACERlab (GE Medical Systems) software. A GE Healthcare BAS-*IP* MS storage phosphor screen 35cm x 43cm was used for radioTLC (Fisher Scientific UK Ltd, Loughborough, UK). Detection and evaluation was performed using a Duerr CR 35 NDT (Raytest Isotopenmessgeraete GmbH, Straubenhardt, Germany) and Raytest AIDA QWBA software. NMR spectra were recorded on a Bruker Avance III 400 QNP Ultrashield Plus Cryo or a Bruker Avance 500 Cryo Ultrashield (Bruker UK Ltd, Coventry, UK). Chemical shifts are reported downfield from TMS, relative to the solvent residual signal. Low-resolution mass spectrometry was conducted using a Bruker Esquire (Bruker UK Ltd, Coventry, UK) electron spray ion source and detector. Accurate high-resolution mass spectra were recorded on an Orbitrap spectrometer using electron spray ionisation. Preparative HPLC and flash chromatography were conducted using a Gilson PLC 2020 chromatography system using normal phase columns and cartridges. Radiosynthesis with ^{11}C was performed on a GE TRACERlab FX C synthesis module modified to allow for captive solvent methylation, radiosyntheses using ^{18}F were conducted using a GE TRACERlab FX F-N synthesis module. GE TRACERlab software was used in both cases for remote controlling of the process.

Synthesis of 4-(2-(4-((1-(4-fluorobenzyl)-1*H*-benzo[*d*]imidazol-2-yl)amino)piperidin-1-yl)ethyl)phenol (2): Astemizole (1 g, 21 mmol) was suspended in 48% aqueous HBr (15 ml) and refluxed for 14 h. The mixture was concentrated to dryness and the residue was taken up in DMF-4 M aqueous KOH (25 ml) and the pH was adjusted to 12. The solids were filtered off and saturated ammonium chloride solution was added (5 ml). The precipitate was collected by filtration and purified via flash chromatography on a grace silica gel cartridge (80 g) using isocratic elution (MeOH-CH_2Cl_2, 1:9) over 30 minutes at 25 ml/min) was used as mobile phase. The final product was obtained in an overall yield of 68% (660 mg, 15 mmol). Analytical data was in accordance with previously published data.

General procedure for synthesis of compounds 4a-e: 100 mg (0.22 mmol) of compound 2 were combined with 70 mg (2 equiv.) of potassium carbonate in acetone (5 ml) and heated to 70 °C in a 15 ml screw cap vial for 14 hours. Subsequently, the solids were filtered off and the residual mixture was concentrated in vacuo. The residue was re-dissolved in CH_2Cl_2 and purified via preparative HPLC on a Macherey-Nagel 250 mm x 40 mm Silica Gel column using gradient elution (MeOH-CH_2Cl_2, 0-3 min 0% MeOH; 3-17 min →10% MeOH; 17-20 min 10% MeOH, 20-21 min →0% MeOH; 21-24 min 0% MeOH).

1-(4-fluorobenzyl)-N-(1-(4-(2-fluoroethoxy)phenethyl)piperidin-4-yl)-1H-benzo[d]imidazol-2-amine (4a): ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.45 (q, J = 11 Hz, 2H), 2.11 (d, J = 12 Hz, 2 H),

2.24 (t, J = 11 Hz, 1H), 2.53 (dd, J = 8 Hz, J = 12 Hz, 2H), 2.71 (dd, J = 8 Hz, J = 12 Hz, 2H), 2.81 (d, J = 10 Hz, 2 H), 3.84-4.05 (m, 1 H), 4.17 (dt, J = 4 Hz, JHF = 28.9 Hz, 2 H), 4.72 (dt, J = 4 Hz, JHF = 47.5 Hz, 2 H), 5.04 (s, 2H), 6.83 (d, J = 9 Hz, 2H), 6.98- 7.15 (m, 8 H), 7.51 (d, J = 8 Hz, 2 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 163.7, 161.2, 156.8, 153.3, 142.3, 134.5, 132.8, 131.2, 131.1, 129.7, 128.3, 128.2, 121.6, 119.8, 116.6, 116.3, 116.1, 114.6, 107.1, 81.9 (d, JHF = 169.5 Hz), 67.3, 67.1, 60.6, 52.1, 49.6, 45.0, 32.8, 32.5. ^{19}F NMR (376 MHz, $CDCl_3$) δ (ppm): -224.0, -113.7. MS (ESI) = 490.3, $C_{29}H_{32}F_2N_4O$ requires 490.2544, HRMS $C_{29}H_{33}F_2N_4O$ requires 491.2617, found: 491.2612 $[M+H]^+$, $C_{29}H_{32}F_2N_4O$ requires C, 70.00; H, 6.57; F, 7.75; N, 11.42; O, 3.26; found C, 70.65; H, 6.52; N, 11.80.

1-(4-fluorobenzyl)-N-(1-(4-(3-fluoropropoxy)phenethyl)piperidin-4-yl)-1H-benzo[d]imidazol-2-amine (4b): ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.51 (q, J = 11 Hz, 2H), 2.12 (d, J = 12 Hz, 2 H), 2.28 (t, J = 11 Hz, 1H), 2.58 (dd, J = 8 Hz, J = 12 Hz, 2H), 2.75 (dd, J = 8 Hz, J = 12 Hz, 2H), 2.86 (d, J = 10 Hz, 2 H), 3.95 (brs, 1 H), 4.06 (t, J = 6 Hz, 2H), 4.62 (dt, J = 4 Hz, JHF = 47Hz, 2 H), 5.06 (s, 2H), 6.81 (d, J = 9 Hz, 2H), 6.98- 7.15 (m, 8 H), 7.50 (d, J = 8 Hz, 2 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 163.7, 161.3, 157.3, 153.3, 142.3, 134.5, 132.0, 131.2, 131.1, 129.6, 128.3, 128.2, 121.6, 119.8, 116.6, 116.3, 116.1, 114.5, 107.2, 80.7 (d, JHF = 163.4 Hz), 63.5, 60.5, 52.1, 49.4, 45.1, 32.3 (d, J = 29 Hz), 30.4 (d, J = 19 Hz). ^{19}F NMR (376 MHz, $CDCl_3$) δ (ppm): -222.5-113.7. MS (ESI) = 504.3, $C_{30}H_{34}F_4N_4O$ requires 504.2701, HRMS $C_{30}H_{35}F_4N_4O$ requires 505.2779, found: $[M+H]^+$, $C_{30}H_{35}F_2N_4O$ requires C, 71.41; H, 6.79; F, 7.53; N, 11.10; O, 3.17; found C, 71.74; H, 7.06; N, 10.92.

1-(4-fluorobenzyl)-N-(1-(4-(4-fluorobutoxy)phenethyl)piperidin-4-yl)-1H-benzo[d]imidazol-2-amine (4c): ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.56 (q, J = 11 Hz, 2H), 1.79-1.93 (m, 4H), 2.12 (d, J = 11.5 Hz, 2 H), 2.31 (t, J = 10 Hz, 1H), 2.59 (dd, J = 8 Hz, J = 11 Hz, 2H), 2.76 (dd, J = 5 Hz, J = 11 Hz, 2H), 2.81 (d, J = 10 Hz, 2 H), 3.52-3.81 (m, 1 H), 3.97 (t, J = 6 Hz, 2 H), 3.99 (m, 2 H), 4.51 (dt, J = 5 Hz, JHF = 47.3 Hz, 2 H), 5.06 (s, 2H), 6.80 (d, J = 9 Hz, 2H), 6.98- 7.16 (m, 8 H), 7.49 (d, J = 8 Hz, 2 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 163.7, 161.2, 157.4, 153.2, 142.2, 134.5, 131.7, 131.2, 131.1, 129.7, 128.3, 128.2, 121.6, 119.8, 116.5, 116.3, 116.1, 114.5, 107.2, 83.8 (d, JHF = 163.6 Hz), 67.3, 60.5, 52.1, 49.4, 45.1, 32.4, 32.3 (d, JHF = 28.5 Hz), 27.2 (d, JHF = 18.5 Hz), 25.2 (d, JHF = 5 Hz). ^{19}F NMR (376 MHz, $CDCl_3$) δ (ppm): -222.2, -113.7. MS (ESI) = 519.3, $C_{31}H_{37}F_2N_4O$ requires 519.2930, HRMS $C_{31}H_{37}F_2N_4O$ requires 519.2930, found: 519.2921 $[M]^+$, $C_{31}H_{37}F_2N_4O$ requires C, 71.79; H, 7.00; F, 7.33; N, 10.80; O, 3.08; found C, 71.87; H, 6.96; N, 10.91.

1-(4-fluorobenzyl)-N-(1-(4-(2-hydroxyethoxy)phenethyl)piperidin-4-yl)-1H-benzo[d]imidazol-2-amine (4d): ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.39 (q, J = 8 Hz, 2H), 1.86 (t, J = 11 Hz, 2 H), 2.03 (d, J = 11 Hz, 2 H), 2.38 (t, J = 8 Hz, 1H), 2.39 (d, J = 6 Hz, 1H), 2.63 (d, J = 6 Hz, 1 H), 2.64 (t, J = 8 Hz, 1H), 2.78 (d, J = 10.5 Hz, 2 H), 3.79-3.96 (m, 4 H), 5.03 (s, 2H), 6.81 (d, J = 9 Hz, 2H), 6.93- 7.15 (m, 8 H), 7.52 (d, J = 8 Hz, 2 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 163.7, 161.3, 157.1, 155.7, 153.1, 141.9, 141.6, 134.4, 134.2, 132,7, 131.0, 130.8, 129.6, 128.3, 128.2, 121.9, 121.7, 120.1, 120.0, 116.5, 116.3, 114.5, 107.3, 69.3, 61.4, 60.6, 52.2, 50.0, 45.1, 32.8, 32.5. MS (ESI) = 488.3, $C_{29}H_{33}FN_4O_2$ requires 488.2588, HRMS $C_{31}H_{34}FN_4O_2$ requires 489.2666, found: 489.2662 $[M]^+$, $C_{29}H_{33}FN_4O_2$ requires C, 71.29; H, 6.81; F, 3.89; N, 11.47; O, 6.55; found C, 71.11; H, 7.02; N, 11.29.

1-(4-fluorobenzyl)-N-(1-(4-(2,2,2-trifluoroethoxy)phenethyl)piperidin-4-yl)-1H-benzo[d]imidazol-2-amine (4e): ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.38 (q, J = 10 Hz, 2H), 1.85 (t, J = 11 Hz, 2H), 2.02 (d, J = 11 Hz, 2 H), 2.37 (t, J = 8 Hz, 1H), 2.39 (d, J = 6 Hz, 1H), 2.62 (d, J = 6 Hz, 1 H), 2.64 (t, J = 8 Hz, 1H), 2.79 (d, J = 11 Hz, 2 H), 3.79-3.95 (m, 2 H), 5.03 (s, 2H), 6.82 (d, J = 9 Hz, 2H), 6.94-7.14 (m, 8 H), 7.52 (d, J = 8 Hz, 2 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 163.7, 161.3, 155.7, 153.1, 141.5, 134.2, 130.8, 130.7, 130.6, 129.6, 129.5, 128.3, 128.2, 121.9, 120.2, 116.4, 116.3, 116.1, 113.9, 107.3, 61.4, 52.2, 50.0, 45.1, 32.7, 32.5, 30.9. ^{19}F NMR (376 MHz, $CDCl_3$) δ (ppm): -77.7, -113.6. MS (ESI) = 526.2, $C_{29}H_{30}F_4N_4O$ requires 526.2356, HRMS $C_{29}H_{30}F_4N_4O$ requires 526.2356, found: 527.2360 [M+H], $C_{29}H_{30}F_4N_4O$ requires C, 66.15; H, 5.74; F, 14.43; N, 10.64; found C, 66.09; H, 5.72, N, 10.86.

1-(4-fluorobenzyl)-N-(1-(4-(2-p-toluenesulfonyloxyethoxy)phenethyl)piperidin-4-yl)-1H-benzo[d]imidazol-2-amine (5): ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.42 (q, J = 8 Hz, 2H), 1.86 (t, J = 11 Hz, 2 H), 2.11 (dd, J = 3 Hz, J = 13 Hz, 2 H), 2.24 (t, J = 10 Hz, 1H), 2.44 (s, CH_3, 3H), 2.51 (t, J = 7 Hz, 1H), 2.53 (d, J = 5 Hz, 1H), 2.73 (d, J = 5 Hz, 1 H), 2.75 (t, J = 7 Hz, 1H), 2.79 (d, J = 9.5

Hz, 2 H), 3.72 (d, J = 8 Hz, 2 H), 3.95 (m, 2H), 5.04 (s, 2H), 6.87 (d, J = 9 Hz, 2H), 6.98- 7.15 (m, 8 H), 7.29 (d, J = 9 Hz, 2 H), 7.51 (d, J = 8 Hz, 2 H), 7.69 (d, J = 9 Hz, 2 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 163.7, 161.3, 153.2, 148.0, 145.2, 142.3, 139.2, 134.5, 132.5, 131.0, 129.8, 129.7, 128.5, 128.3, 122.3, 121.6, 119.9, 116.6, 116.4, 116.1, 107.1, 60.6, 52.1, 49.6, 45.1, 33.1, 32.6, 21.7. MS (ESI) = 642.3, $C_{36}H_{39}FN_4O_4S$ requires 642.3, HRMS $C_{36}H_{40}FN_4O_4S$ requires 643.2749, found: 643.2737 $[M+H]^+$, $C_{36}H_{40}FN_4O_4S$ requires C, 67.27; H, 6.12; F, 2.96; N, 8.72; O, 9.96; S, 4.99; found C, 66.79; H, 5.72, N, 8.86.

Synthesis of [^{11}C]1: An aluminium target filled with $^{14}N_2$ containing 2% O_2 was irradiated with protons at an incident energy of 13 MeV (13→3 MeV) and a beam current of 20-30 µA for 15-30 minutes using a GE PETtrace cyclotron to produce [^{11}C]CO_2 using the $^{14}N(p,\alpha)^{11}C$ nuclear reaction. The product was converted into [^{11}C]CH_3I using a GE MeI Microlab synthesiser. [^{11}C]CH_3I was released in a stream of helium and passed directly through a 3 ml stainless steel HPLC loop containing compound **2** (0.3 mg) and 40% TBAOH (2 µl) in DMF (300 µl) for 2 minutes after which the contents of the loop were injected onto a Merck Chromolith RP18e column (4.6x100 mm) for purification. The product was eluted using 40% MeCN in 50 mM ammonium formate solution at a flow rate of 4 ml/minute. Formulation was achieved via dilution of the product fraction in water (40 ml), trapping of the radioactive product on an Oasis HLB cartridge and elution of the product in EtOH (1 ml) followed by water (11 ml). Via this procedure [^{11}C]**1** was obtained in a concentration of approximately 1 nmol/ml, a radiochemical purity of >99% in a non-decay corrected yield of 3-5%, 35 minutes from EOB.

Synthesis of [^{18}F]4a: A silver target filled with $H_2{}^{18}O$ (Rotem, Israel) was irradiated with protons at an incident energy of 16 MeV (16→3MeV) and a beam current of 30-40 µA for 5 to 20 minutes using a GE PETtrace cyclotron to produce [^{18}F]Fluoride ion *via* the $^{18}O(p,n)^{18}F$ nuclear reaction. The radionuclide was extracted from the target water via solid phase extraction on a Waters accell plus light QMA strong anion exchanger cartridge (CO_3^{2-}-form). Reactive [^{18}F]F^- was obtained after elution of the trapped radioactivity using a mixture of crypt-222 (22 mg) in acetonitrile (300 µl) and K_2CO_3 (7 mg) in water (300 µl). The eluate was collected in the reaction vessel of a GE FX F-N radiosynthesis module and the volatiles were evaporated by heating at 95 ºC for 6 min in a constant stream of helium and under reduced pressure. During this step, 3 portions of acetonitrile (1ml in total) were added to the reactor to promote the removal of residual water. At the end of this step, the reactor was cooled to 60 ºC and ethylene ditosylate (8 mg in 800 µl of MeCN) was added to the dry crypt-222/K_2CO_3/^{18}F residue. The reaction vessel was sealed and heated to 90 ºC for 15 minutes after which the reaction mixture was cooled to 60 ºC. Compound **2** (12 mg in 1 ml DMF) together with 30 µl KOH 10M were added to the reactor and the resulting mixture was heated to 120 ºC for 20 minutes. The reactor was cooled to 50 ºC and 1.5 ml of HPLC mobile phase was added to the reaction mixture. The mixture was purified by HPLC (see **general** section for details). [^{18}F]**4a** was collected in a flask containing 40 ml of water after a retention time of 26-29 minutes. The diluted product fraction was passed through an Oasis HLB (30 mg) cartridge. After rinsing of the cartridge with 10 ml of water, the product was eluted from the resin with 300 µl EtOH. The final formulation was obtained by diluting an appropriate aliquot of the product solution with saline.

Radioligand displacement assay: A stock solution of [^{3}H]astemizole (10 nmol/ml) in EtOH was used for all data points. Eleven dilutions (5-fold final concentration) of the test compounds were prepared in 8% EtOH (0.2 nM, 0.5 nM, 1 nM, 2 nM, 5 nM, 10 nM, 20 nM, 50 nM, 100 nM, 200 nM and 0.5 µM. Aliquots (50 µl) of radioligand were dispensed into the wells of a 96-well plate containing 100 µl of 8% EtOH solution. Then 50-µl aliquots of the test compound dilutions were added. 4 compounds were analysed in duplicate per plate (88 wells were used in total). Three of the remaining wells were charged with 50 µl of a 5 µM lansoprazole solution (non-specific binding) and three wells were charged with 8% EtOH solution instead of a test substance (maximum uptake). Finally, 50 µl of a solution of tau-aggregates (1 µg per well) were added to each of the wells. The two remaining wells were charged with 150 µl of standard binding solution instead of tau-stock solution (blank).
The final volume (250 µl) was incubated at room temperature for 1 hours, then harvested by filtration onto 96-well filter plates (GE/Whatman Unifilter 96/350, GF/C membrane). 3 rapid 300 µl washing cycles were performed with chilled 8% ethanol.

Synthesis of htau fibrils: Synthetic fibrils were prepared via incubation of soluble recombinant htau-441 with lithium heparin (4.4 µM) and dithiothreitol (1 mM, DTT). Lyophilised htau was reconstituted

in water to obtain a concentration of 1 mg/ml (21.8 μM) in 50 mM MES pH 6.8, 100 mM NaCl and 0.5 mM EGTA. Incubation for fibril synthesis was conducted at 4.4 μM tau concentration. Protein stock solution was diluted with 50 mM MES pH 6.8, 100 mM NaCl and 0.5 mM EGTA. The vial was tightly capped and the solution was incubated at 37 °C for 7 days. The vial was vortexed on days 1 and 3. Formation of fibrilar tau aggregates was controlled using thioflavin T fluorescence.

PET imaging: All animal experiments were conducted in accordance with the UK Animal (Scientific Procedures) Act of 1986 (Project licence: 80/2234). Studies were performed in Wistar rats (Charles River, Margate, Kent). Anaesthesia was induced with 2% isoflurane administered in 1 l/min oxygen and maintained throughout with 1% isoflurane. The tail vein was cannulated for intravenous administration of [^{18}F]FEAST and cyclosporine A. The heart was cannulated *post-mortem* to allow for collection of a blood sample. During all surgical procedures body temperature was maintained at 37°C using a heating blanket connected to a rectal thermo-probe. Following PET scanning, the animals were sacrificed by intra-venous injection of 1 mL (200 mg/mL) pentobarbital sodium (Euthanal) and brains were harvested to carry out metabolite analysis.

PET data were acquired using a microPET Focus 220 scanner (Concorde Microsystems, Knoxville, TN, USA). The rats were placed prone on the scanner bed and the head fixed in a custom made plastic frame using ear bars and a bite bar. Anaesthesia and body temperature was maintained as described above. In addition, oxygen saturation, heart rate and respiratory rate were measured and maintained within physiological limits throughout using a non-invasive mouseOX™ (Starr Life Science Corp, Oakmont, PA, USA) pulse oximeter sensor attached to the foot.

Before injection, singles-mode transmission data were acquired for 8.5 mins using a rotating ^{68}Ge/^{68}Ga point source (~20 MBq). An attenuation correction sinogram was produced from this scan and a blank scan of the same duration, with scatter correction applied. In all experiments [^{18}F]FEAST was injected intravenously over 30 seconds, followed by a 15 second heparinised saline flush. List-mode data were histogrammed into sinograms for the following time frames: 12 × 5s, 6 × 10s, 3 x 20s, 4 x 30s, 5 x 1min, 10 x 2min, 24 x 5min (2.5h in total). Corrections were applied for random events, dead time, normalisation, attenuation, scatter and decay. Fourier rebinning (*16*) was used to compress the 4D sinograms to 3D prior to reconstruction with 2D filtered backprojection with a Hann window cut-off at the Nyquist frequency. The image voxel size was 0.95 x 0.95 x 0.80mm, with an array size of 128 x 128 x 95. The reconstructed images were converted to kBq/mL using global and slice factors determined from imaging a uniform phantom filled with a [^{18}F]fluoride solution. This phantom acquisition was also used to cross-calibrate the scanner and the well counter used to measure blood radioactivity concentration.

Metabolite Analysis: Blood samples were collected 150 minutes post-injection and used for metabolite studies. 500 μl blood samples were centrifuged at 5000 rpm for 5 minutes, then 250 μl of the supernatant plasma was transferred into 500 μl of methanol, thoroughly agitated and centrifuged at 5000 rpm for 5 minutes. An aliquot (50 μl) of the supernatant was counted and 20μl of the supernatant was directly injected into the analytical HPLC system. The HPLC eluate was fractioned into two fractions containing the radioactive metabolites and intact [^{18}F]FEAST, respectively. Both fractions were weighed and an aliquot of each fraction was counted using a Hidex 431-040 well counter (Hidex Oy, Turku, Finland). The supernatant was directly spotted on Merck silica gel 60 coated aluminium plates for analysis.

Brain tissue was homogenised by grinding under methanol (3 mL/g of tissue) in a glass tissue homogeniser for 5 minutes. The supernatant layer was transferred into a centrifuge tube and centrifuged at 5000 rpm for 5 minutes. The supernatant was analysed in the same manner as described for the blood analysis.

PET Data Analysis: For each scan a mean PET image (1-2.5h post-injection) was manually co-registered to a T2-weighted MR atlas of the rat brain (http://www.loni.ucla.edu/Atlases/Atlas_Detail.jsp?atlas_id=1/Rat.html) and the dynamic PET images were resliced to the MR atlas using these co-registration parameters. To facilitate the production of regional time-activity curves (TACs), the following regions of interest (ROIs) were manually defined on the MR atlas using Analyze 7.0 software (AnalyzeDirect, Overland Park, KS, USA): frontal cortex, striatum, hippocampus, and thalamus. Image values (kBq/ml) were converted to standardized uptake value (SUV; normalization by weight), and SUV maps (120-150min post-injection) and ROI SUV TACs were generated.

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