Supporting Information

Structure-activity and Brain Kinetics Relationships of ^{18}F-Labeled Benzimidazopyridine Derivatives as Tau PET Tracers

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Material and Methods

General remarks

All reagents were commercial products used without further purification unless indicated otherwise. All compounds were purified by Smart Flash EPCLC W-Prep 2XY (Yamazen Corporation, Osaka, Japan) unless otherwise stated. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL JNM-ECS400 (JEOL, Tokyo, Japan) with tetramethylsilane as an internal standard. Coupling constants are reported in Hertz. Multiplicity was defined as singlet (s), doublet (d), doublet of doublets (dd), triplet (t), or multiplet (m). ESI mass spectrometry was conducted with a Shimadzu LCMS-2020 EV (Shimadzu, Kyoto, Japan). High-resolution mass spectrometry (HRMS) was carried out with a JEOL JMS-700 (JEOL). Reversed-phase high performance liquid chromatography (HPLC) was performed with a Shimadzu system (an LC-20AD pump with SPD-20A UV detector, λ = 254 nm) using a Cosmosil C_{18} column (5C_{18}-AR-II or 5C_{18}-MS-II, 4.6 mm I.D.×150 mm, Nacalai Tesque, Kyoto, Japan) and acetonitrile/H_2O (with 0.1% trifluoroacetic acid (TFA) or 0.1% triethylamine (Et_3N)) as the mobile phase at a flow rate of 1.0 mL/min. All key compounds were proven by this method to show >95% purity.

Chemistry

*7-Bromo-N,N-(2-fluoroethyl)(methyl)amino pyrido[1,2-a]benzimidazole (**1**)*

A mixture of 2-bromo-*N*,*N*-(2-fluoroethyl)(methyl)pyridine-4-amine (794.6 mg, 3.41 mmol), 2,5-dibromoaniline (855.6 mg, 3.41 mmol), CuI (129.9 mg, 0.682 mmol), Cs_2CO_3 (3333 mg, 10.23 mmol), and 1,10-phenanthroline (245.1 mg, 1.36 mmol) in xylene (20.0 mL) was stirred under reflux for 24 h. The mixture was extracted with chloroform, and the organic phase was separated and dried over Na_2SO_4. The solvent was removed and the residue was purified by silica gel chromatography (chloroform/methanol=99/1) to give 489.3 mg of 3 (45.0%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (d, J = 7.6 Hz, 1H), 7.83 (s, 1H), 7.49 (d, J = 8.4 Hz, 1H), 7.25 (d, J = 8.4 Hz, 1H), 6.52-6.47 (m, 2H), 4.71 (t, J = 5.2 Hz, 1H), 4.59 (t, J = 5.2 Hz, 1H), 3.78 (t, J = 5.2 Hz, 1H), 3.71 (t, J = 5.2 Hz, 1H), 3.13 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.2, 149.9, 146.8, 125.1, 121.8, 120.6, 118.0, 110.2, 102.3, 91.3, 82.3, 80.6, 52.5, 39.0. HRMS (FAB) *m/z* calcd. for $C_{14}H_{13}BrFN_3$ (MH^+) 322.0355, found 322.0359.

*7-Chloro-N,N-(2-fluoroethyl)(methyl)amino pyrido[1,2-a]benzimidazole (**2**)*

The same reaction described above to prepare **1** was used, and 11.0 mg of **2** was obtained from 2-bromo-5-chloroaniline and 2-bromo-*N*,*N*-(2-fluoroethyl)(methyl)pyridine-4-amine in a yield of 13.3%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (d, J = 7.6 Hz, 1H), 7.69 (d, J = 1.6 Hz, 1H), 7.59 (d, J = 8.4 Hz, 1H), 7.15 (dd, J = 2.0, 8.4 Hz, 1H), 6.57 (dd, J = 2.0, 7.2 Hz, 1H), 6.53 (d, J = 2.4 Hz, 1H), 4.73 (t, J = 5.2 Hz, 1H), 4.61 (t, J = 5.2 Hz, 1H), 3.81 (t, J = 5.2 Hz, 1H), 3.74 (t, J = 5.2 Hz, 1H), 3.67 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.4, 149.8, 130.4, 125.2, 119.2, 117.7, 109.9, 102.3, 91.4, 82.3, 80.6, 52.5, 39.1, 29.3. HRMS (FAB) *m/z* calcd. for $C_{14}H_{13}ClFN_3$ (MH^+) 278.0860, found 278.0858.

*7-Methyl-N,N-(2-fluoroethyl)(methyl)amino pyrido[1,2-a]benzimidazole (**3**)*

The same reaction described above to prepare **1** was used, and 28.5 mg of **3** was obtained from 2-bromo-5-methylaniline and 2-bromo-*N*,*N*-(2-fluoroethyl)(methyl)pyridine-4-amine in a yield of 14.0%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (d, J = 7.6, 1H), 7.56-7.51 (m, 2H), 7.02 (d, J = 8.0 Hz, 1H), 6.48-6.53 (m, 2H), 4.71 (t, J = 4.8 Hz, 1H), 4.59 (t, J = 4.8 Hz, 1H), 3.76 (t, J = 4.8 Hz, 1H), 3.67 (t, J = 4.8 Hz, 1H), 3.12 (s, 3H), 2.51 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.7, 135.0, 125.2, 120.8, 117.5, 108.8, 101.9, 91.1, 82.3, 80.6, 52.5, 39.1, 30.2, 21.9. HRMS (FAB) *m/z* calcd. for $C_{15}H_{17}FN_3$ (MH^+) 258.1407, found 258.1411.

*7-Methoxy-N,N-(2-fluoroethyl)(methyl)amino pyrido[1,2-a]benzimidazole (**4**)*

The same reaction described above to prepare **1** was used, and 26.4 mg of **4** was obtained from 2-bromo-5-methoxylaniline and 2-bromo-*N*,*N*-(2-fluoroethyl)(methyl)pyridine-4-amine in a yield of 15.0%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (d, J = 7.2 Hz, 1H), 7.54 (d, J = 4.0 Hz, 1H), 7.19 (d, J = 2.0 Hz, 1H), 6.81 (dd, J = 2.4, 8.8 Hz, 1H), 6.51-6.45 (m, 2H) 4.70 (t, J = 4.8 Hz, 1H), 4.59 (t, J = 4.8 Hz, 1H), 3.90 (s, 3H), 3.76 (t, J = 4.8 Hz, 1H), 3.70 (t, J = 4.8 Hz, 1H), 3.12 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.1, 149.2, 125.0, 109.6, 108.7, 101.6, 100.2, 91.4, 82.4, 80.7, 77.2, 55.7, 52.4, 39.1. HRMS (FAB) *m/z* calcd. for $C_{15}H_{17}FN_3O$ (MH^+) 274.1356, found 274.1351.

*N,N-(2-Fluoroethyl)(methyl)amino pyrido[1,2-a]benzimidazole (**5**)*

The same reaction described above to prepare **1** was used, and 143.1 mg of **5** was obtained from 2-bromo-aniline and 2-bromo-*N*,*N*-(2-fluoroethyl)(methyl)pyridine-4-amine in a yield of 32.0%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (d, J = 8.4 Hz, 1H), 7.74 (d, J = 7.6 Hz, 1H), 7.69 (d, J = 7.6 Hz, 1H), 7.41 (t, J = 7.2 Hz, 1H), 7.20 (t, J = 7.2 Hz, 1H), 6.53-6.50 (m, 2H), 4.71 (t, J = 5.2 Hz, 1H),

4.60 (t, J = 5.2 Hz, 1H), 3.77 (t, J = 5.2 Hz, 1H), 3.71 (t, J = 5.2 Hz, 1H), 3.14 (s, 3H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 151.3, 149.4, 145.3, 128.5, 125.0, 119.0, 117.9, 109.2, 101.8, 91.2, 82.3, 80.6, 52.3, 38.9. HRMS (FAB) *m/z* calcd. for $C_{14}H_{15}FN_3$ (MH^+) 244.1250, found 244.1254.

*7-Bromo-N,N-(2-((tert-butyldimethylsilyl)oxy)ethyl)(methyl)aminopyrido[1,2-a]benzimidazole (**6**)*

The same reaction described above to prepare **1** was used, and 74.7 mg of **6** was obtained from 2,5-dibromoaniline and 2-bromo-*N*,*N*-(2-((tert-butyldimethylsilyl)oxy)ethyl)(methyl)pyridine-4-amine in a yield of 5.0%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (d, J = 8.0 Hz, 1H), 7.82 (d, J = 2.0 Hz, 1H), 7.50 (d, J = 8.4 Hz, 1H), 7.24 (dd, J =2.0, 8.4 Hz, 1H), 6.55 (dd, J = 2.4, 7.6 Hz, 1H), 6.44 (d, J = 2.4 Hz, 1H), 3.82 (t, J = 5.6 Hz, 2H), 3.58 (t, J = 5.6 Hz, 2H), 3.12 (s, 3H), 0.85 (s, 9H), 0.00 (s, 6H). MS (ESI) *m/z* calcd. for $C_{20}H_{28}BrN_3OSi$ (MH^+) 436.1, found 435.9.

*7-Chloro-N,N-(2-((tert-butyldimethylsilyl)oxy)ethyl)(methyl)aminopyrido[1,2-a]benzimidazole (**7**)*

The same reaction described above to prepare **1** was used, and 58.9 mg of **7** was obtained from 2-bromo-5-chloroaniline and 2-bromo-*N*,*N*-(2-((tert-butyldimethylsilyl)oxy)ethyl)(methyl)pyridine-4-amine in a yield of 17.0%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (d, J = 7.6 Hz, 1H), 7.65 (d, J = 1.6 Hz, 1H), 7.54 (d, J = 8.4 Hz, 1H), 7.10 (dd, J = 1.2, 8.4 Hz, 1H), 6.58 (dd, J = 2.0, 8.8 Hz, 1H), 6.45 (d, J = 2.0 Hz, 1H), 3.82 (t, J = 5.6 Hz, 2H), 3.57 (t, J = 5.6 Hz, 2H), 3.10 (s, 3H), 0.85 (s, 9H), 0.00 (s, 6H). MS (ESI) *m/z* calcd. for $C_{20}H_{28}ClN_3OSi$ (MH^+) 390.2, found 390.3 [MH^+].

*7-Methyl-N,N-(2-((tert-butyldimethylsilyl)oxy)ethyl)(methyl)aminopyrido[1,2-a]benzimidazole (**8**)*

The same reaction described above to prepare **1** was used, and 31.1 mg of **8** was obtained from 2-bromo-5-methylaniline and 2-bromo-*N*,*N*-(2-((tert-butyldimethylsilyl)oxy)ethyl)(methyl)pyridine-4-amine in a yield of 5.0%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (d, J = 7.6 Hz, 1H), 7.57-7.54 (m, 2H), 7.03 (d, J = 8.0 Hz, 1H), 6.63 (dd, J = 1.6, 8.0 Hz, 1H), 6.59 (s, 1H), 3.83 (t, J = 5.2 Hz, 2H), 3.59 (t, J = 5.2 Hz, 2H), 3.12 (s, 3H), 2.50 (s, 3H), 0.85 (s, 9H), 0.00 (s, 6H). MS (ESI) *m/z* calcd. for $C_{21}H_{31}N_3OSi$ (MH^+) 370.2, found 370.4 [MH^+].

*7-Methoxy-N,N-(2-((tert-butyldimethylsilyl)oxy)ethyl)(methyl)aminopyrido[1,2-a]benzimidazole (**9**)*

The same reaction described above to prepare **1** was used, and 52.1 mg of **9** was obtained from 2-bromo-5-methoxylaniline and 2-bromo-*N*,*N*-(2-((tert-butyldimethylsilyl)oxy)ethyl)(methyl)pyridine-4-amine in a yield of 8.0%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.67 (d, J = 7.2 Hz, 1H), 7.54 (d, J = 8.8 Hz, 1H), 7.19 (d, J = 2.0 Hz, 1H), 6.79 (dd, J = 2.4, 8.8 Hz, 1H), 6.55 (dd, J = 2.4, 7.6 Hz, 1H), 6.47 (d, J = 2.0 Hz, 1H), 3.89 (s, 3H), 3.83 (t, J = 5.2 Hz, 2H), 3.57 (t, J = 5.2 Hz, 2H), 3.11 (s, 3H), 0.85 (s, 9H), 0.00 (s, 6H). MS (ESI) *m/z* calcd. for $C_{21}H_{31}N_3O_2Si$ (MH^+) 386.2, found 386.1 [MH^+].

*N,N-(2-((tert-Butyldimethylsilyl)oxy)ethyl)(methyl)aminopyrido[1,2-a]benzimidazole (**10**)*

The same reaction described above to prepare **1** was used, and 25.4 mg of **10** was obtained from 2-bromoaniline and 2-bromo-*N*,*N*-(2-((tert-butyldimethylsilyl)oxy)ethyl)(methyl)pyridine-4-amine in a yield of 9.0%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (d, J = 7.6 Hz, 1H), 7.73 (d, J = 8.0 Hz, 1H), 7.68 (d, J = 8.0 Hz, 1H), 7.39 (t, J = 7.6 Hz, 1H), 7.19 (t, J = 7.6 Hz, 1H), 6.60 (d, J = 7.6 Hz, 1H), 6.52 (s, 1H), 3.83 (t, J = 5.6 Hz, 2H), 3.59 (t, J = 5.6 Hz, 2H), 3.12 (s, 3H), 0.85 (s, 9H), 0.00 (s, 6H). MS (ESI) *m/z* calcd. for $C_{20}H_{29}N_3OSi$ (MH^+) 356.2, found 356.4 [MH^+].

*7-Bromo-N,N-(2-hydroxyethyl)(methyl)amino pyrido[1,2-a]benzimidazole (**11**)*

To a solution of **6** (74.7 mg, 0.17 mmol) in tetrahydrofuran (THF) (5.00 mL) was added tetra-n-butylammonium fluoride (TBAF) (1 M in THF, 210 μL). The mixture was stirred at room temperature for 3.5 h. The solvent was removed and the residue was purified by silica gel chromatography (chloroform/methanol=6/1) to give 52.7 mg of **11** (97.0%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.73 (d, J = 7.6 Hz, 1H), 8.00 (d, J = 8.4 Hz, 1H), 7.65 (d, J = 1.6 Hz, 1H), 7.24 (dd, J = 2.0, 8.4 Hz, 1H), 6.84 (dd, J = 2.4, 7.6 Hz, 1H), 6.35 (d, J = 2.0 Hz, 1H), 4.77 (t, J = 4.8 Hz, 1H), 3.62-3.55 (m, 4H), 3.08 (s, 3H). MS (ESI) *m/z* calcd. for $C_{14}H_{14}BrN_3O$ (MH^+) 320.0, found 320.1 [MH^+].

*7-Chloro-N,N-(2-hydroxyethyl)(methyl)amino pyrido[1,2-a]benzimidazole (**12**)*

The same reaction described above to prepare **11** was used, and 38.8 mg of **12** was obtained from **7** in a yield of 94.0%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.76 (d, J = 7.6 Hz, 1H), 8.06 (d, J = 8.8 Hz, 1H), 7.52 (d, J = 2.0 Hz, 1H), 7.15 (dd, J = 2.0, 8.4 Hz, 1H), 6.86 (dd, J = 2.4, 7.6 Hz, 1H), 6.36 (d, J = 2.0 Hz, 1H), 4.83 (s, 3H), 3.61-3.57 (m, 4H), 3.08 (s, 3H). MS (ESI) *m/z* calcd. for $C_{14}H_{14}ClN_3O$ (MH^+) 276.1, found 276.1 [MH^+].

*7-Methyl-N,N-(2-hydroxyethyl)(methyl)amino pyrido[1,2-a]benzimidazole (**13**)*

The same reaction described above to prepare **11** was used, and 23.0 mg of **13** was obtained from **8** in a yield of 60.0%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (d, J = 7.6 Hz, 1H), 7.47 (s, 1H), 7.38 (d, J = 8.0 Hz, 1H), 7.00 (d, J = 8.0 Hz, 1H), 6.49-6.46 (m, 2H), 3.92 (t, J = 5.2 Hz, 2H), 3.59 (t, J = 5.2 Hz, 2H), 3.03 (s, 3H), 2.51 (s, 3H). MS (ESI) *m/z* calcd. for $C_{15}H_{17}N_3O$ (MH^+) 256.1, found 256.1 [MH^+].

*7-Methoxy-N,N-(2-hydroxyethyl)(methyl)amino pyrido[1,2-a]benzimidazole (**14**)*

The same reaction described above to prepare **11** was used, and 27.7 mg of **14** was obtained from **9** in a yield of 100%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (d, J = 7.6 Hz, 1H), 7.47 (d, J = 8.4 Hz, 1H), 7.18 (d, J = 2.4 Hz, 1H), 6.81 (dd, J = 2.4, 8.8 Hz, 1H), 6.52-6.50 (m, 2H), 3.93-3.90 (m, 5H), 3.61 (t, J = 6.0 Hz, 2H), 3.08 (s, 3H). MS (ESI) *m/z* calcd. for $C_{15}H_{17}N_3O_2$ (MH^+) 272.3, found 272.3 [MH^+].

*N,N-(2-Hydroxyethyl)(methyl)amino pyrido[1,2-a]benzimidazole (**15**)*

The same reaction described above to prepare **11** was used, and 21.7 mg of **15** was obtained from **10** in a yield of 100%. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.70 (d, J = 7.6 Hz, 1H), 8.01 (d, J = 8.4 Hz, 1H), 7.57-7.49 (m, 2H), 7.40-7.36 (m, 1H), 7.07 (dd, J = 2.8, 8.0 Hz, 1H), 6.57 (d, J = 2.8 Hz, 1H), 3.83 (t, J = 5.2 Hz, 2H), 3.71 (t, J = 5.2 Hz, 2H), 3.22 (s, 3H). MS (ESI) *m/z* calcd. for $C_{14}H_{15}N_3O$ (MH^+) 242.1, found 242.4 [MH^+].

*7-Bromo-N,N-(methyl)(2-((toluenesulfonyl)oxy)ethyl)amino pyrido[1,2-a]benzimidazole (**16**)*

To a solution of **11** (29.2 mg, 0.091 mmol) in dichloromethane (10 mL) were added *p*-toluenesulfonyl chloride (87.7 mg, 0.46 mmol), *N*,*N*-dimethylpyridine-4-amine (DMAP) (11.1 mg, 0.091 mmol), and trimethylamine (63.8 μL, 0.46 mmol) at 0°C. The mixture was stirred at room temperature for 17 h. The mixture was extracted with chloroform, and the organic phase was separated and dried over Na_2SO_4. The solvent was removed and the residue was purified by silica gel chromatography (chloroform/methanol=6/1) to give 35.2 mg of **16** (81.0%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, J = 7.6 Hz, 1H), 7.82 (d, J = 1.6 Hz, 1H), 7.66 (d, J = 8.0 Hz, 2H), 7.50 (d, J = 8.4 Hz, 1H), 7.27-7.25 (m, 1H), 7.18 (d, J = 8.0 Hz, 2H), 6.42 (dd, J = 2.4, 7.6 Hz, 1H), 6.31 (d, J = 2.4 Hz, 1H), 4.22 (t, J = 5.2 Hz, 2H), 3.72 (t, J = 5.2 Hz, 2H), 2.99 (s, 3H), 2.27 (s, 3H). MS (ESI) *m/z* calcd. for $C_{21}H_{20}BrN_3O_3S$ (MH^+) 474.0, found 474.1 [MH^+].

*7-Chloro-N,N-(methyl)(2-((toluenesulfonyl)oxy)ethyl)amino pyrido[1,2-a]benzimidazole (**17**)*

The same reaction described above to prepare **16** was used, and 39.8 mg of **17** was obtained from **12** in a yield of 84.0%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (d, J = 7.6 Hz, 1H), 7.67-7.64 (m, 3H), 7.58-7.56 (m, 1H), 7.18 (d, J = 8.0 Hz, 2H), 7.13 (dd, J = 2.0, 8.8 Hz, 1H) 6.44 (dd, J = 2.0, 7.6 Hz, 1H), 6.32 (d, J = 2.8 Hz, 1H), 4.23 (t, J = 5.2 Hz, 2H), 3.72 (t, J = 5.2 Hz, 2H), 3.00 (s, 3H), 2.27 (s, 3H). MS (ESI) *m/z* calcd. for $C_{21}H_{20}ClN_3O_3S$ (MH^+) 430.1, found 430.1 [MH^+].

*7-Methyl-N,N-(methyl)(2-((toluenesulfonyl)oxy)ethyl)amino pyrido[1,2-a]benzimidazole (**18**)*

The same reaction described above to prepare **16** was used, and 7.2 mg of **18** was obtained from **13** in a yield of 57.0%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (d, J = 7.6 Hz, 1H), 7.68 (d, J = 8.0 Hz, 2H), 7.59 (d, J = 8.0 Hz, 1H), 7.53 (s, 1H), 7.21 (d, J = 8.0 Hz, 2H), 7.08 (d, J = 8.0 Hz, 1H), 6.64 (s, 1H), 6.54 (d, J = 5.2 Hz, 1H), 4.25 (t, J = 5.2 Hz, 2H), 3.77 (t, J = 5.2 Hz, 2H), 3.05 (s, 3H), 2.52 (s, 3H), 2.30 (s, 3H). MS (ESI) *m/z* calcd. for $C_{22}H_{23}N_3O_3S$ (MH^+) 410.1, found 410.3 [MH^+].

*7-Methoxy-N,N-(methyl)(2-((toluenesulfonyl)oxy)ethyl)amino pyrido[1,2-a]benzimidazole (**19**)*

The same reaction described above to prepare **16** was used, and 3.9 mg of **19** was obtained from **14** in a yield of 50.0%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (d, J = 8.4 Hz, 1H), 7.68 (d, J = 8.0 Hz, 2H), 7.57 (d, J = 8.8 Hz, 1H), 7.21-7.18 (m, 3H), 6.85 (dd, J = 1.6, 8.2 Hz, 1H), 6.47-6.43 (m, 2H), 4.25 (t, J = 5.2 Hz, 2H), 3.91 (s, 3H), 3.75 (t, J = 5.2 Hz, 2H), 3.03 (s, 3H), 2.28 (s, 3H). MS (ESI) *m/z* calcd. for $C_{22}H_{23}ClN_3O_4S$ (MH^+) 426.1, found 426.1 [MH^+].

*N,N-(Methyl)(2-((toluenesulfonyl)oxy)ethyl)amino pyrido[1,2-a]benzimidazole (**20**)*

The same reaction described above to prepare **15** was used, and 17.3 mg of **20** was obtained from **15** in a yield of 44.0%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (d, J = 7.2 Hz, 1H), 7.74-7.67 (m, 4H), 7.41 (t, J = 7.2 Hz, 1H), 7.23-7.16 (m, 3H), 6.45-6.41 (m, 2H), 4.23 (t, J = 5.6 Hz, 2H), 3.73 (t, J = 5.6 Hz, 2H), 3.00 (s, 3H), 2.25 (s, 3H). MS (ESI) *m/z* calcd. for $C_{21}H_{21}N_3O_3S$ (MH^+) 396.1, found 396.2 [MH^+].

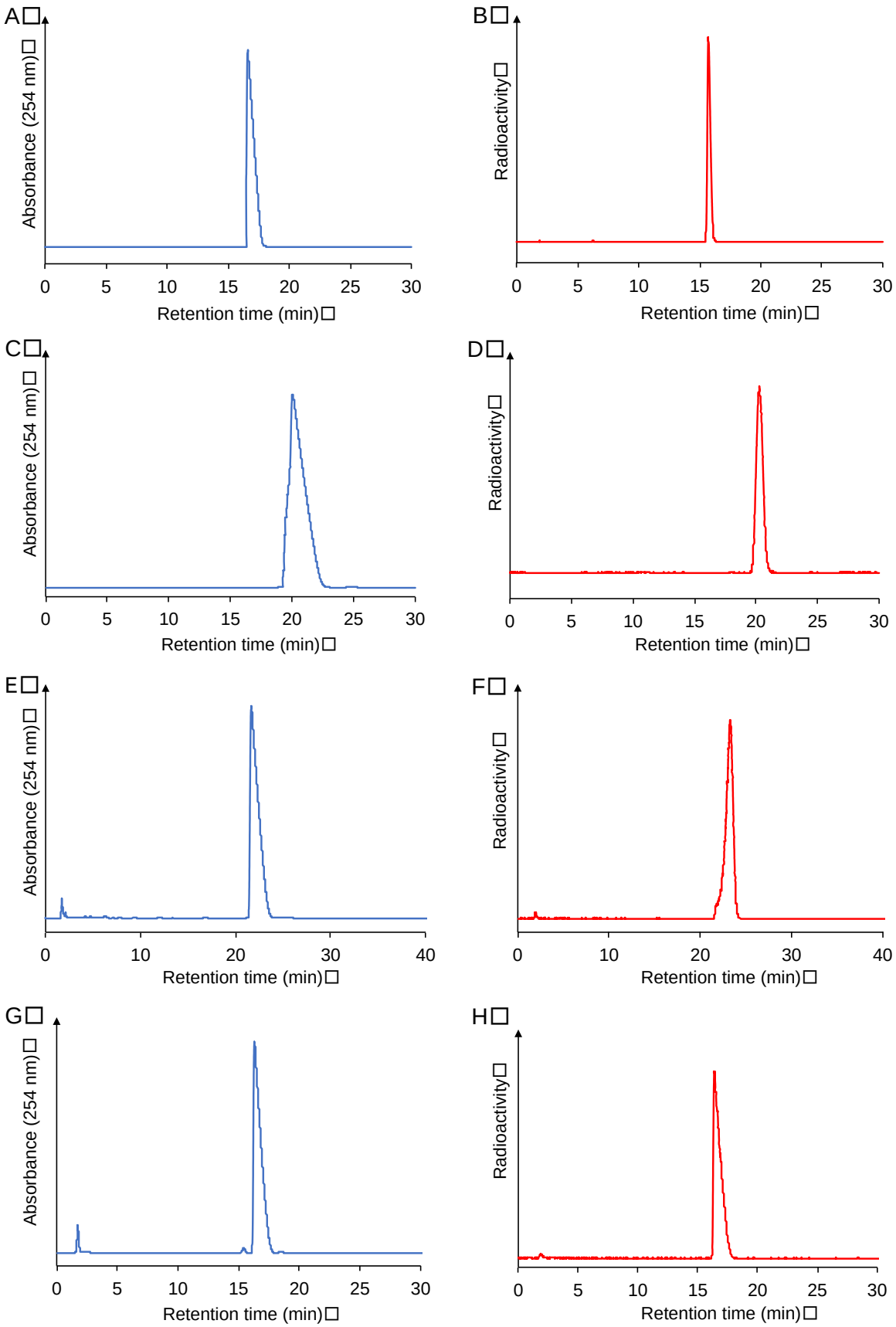
^{18}F-labeling reaction for BIP derivatives

We produced [^{18}F]fluoride in the reaction vessel according to the method reported previously.[2] Kryptofix222 (8.0 mg) was added to the reaction vessel, and the mixture was evaporated azeotropically with dry acetonitrile (300 μL) under a nitrogen gas stream at 120 °C, three times. A solution of Kryptofix222 (1.5 mg) and one of the tosyl precursors (1.0 mg) in anhydrous dimethyl sulfoxide (DMSO) (200 μL) was added to the reaction vessel. The mixture was heated at 100 °C for 20 min. After passing through the Cosmonice Filter (S) (0.45 μm, 4 mm) (Nacalai Tesque), the crude ^{18}F-labeled probes were purified by HPLC on a Cosmosil 5C_{18}-AR-II column with an isocratic solvent of acetonitrile/H_2O/TFA at a flow rate of 1.0 mL/min.

Table S1. Radiochemical yield and purity and specific activity of ^{18}F-labeled BIP derivatives

Compound	Radiochemical yield (%)*	Radiochemical purity (%)	Specific activity (MBq/mmol)
[^{18}F]**1**	65	>99	>8.4
[^{18}F]**2**	60	>99	>695.5
[^{18}F]**3**	53	>99	>20.0
[^{18}F]**4**	37	>99	>38.6
[^{18}F]**5**	51	>99	>41.3

*Data are mean of two independent experiments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Absorbance (254 nm)
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Retention time (min)
B
Radioactivity
0 5 10 15 20 25 30
Retention time (min)
C
Absorbance (254 nm)
0 5 10 15 20 25 30
Retention time (min)
D
Radioactivity
0 5 10 15 20 25 30
Retention time (min)
E
Absorbance (254 nm)
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Retention time (min)
F
Radioactivity
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G
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Retention time (min)
H
Radioactivity
0 5 10 15 20 25 30
Retention time (min)

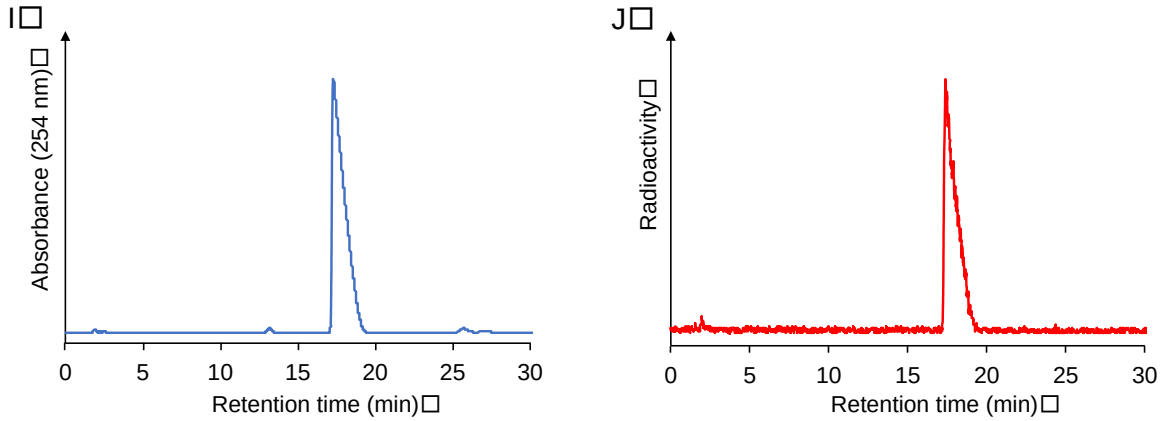


Figure S1. Representative HPLC profiles. UV chromatogram at 254 nm of **1** (A), **2** (C), **3** (E), **4** (G), and **5** (I). Radio-chromatogram of [^{18}F]**1** (B), [^{18}F]**2** (D), [^{18}F]**3** (F), [^{18}F]**4** (H), and [^{18}F]**5** (J). These data were obtained under the following HPLC mobile phase conditions: MeCN (0.1% TFA) / H_2O (0.1% TFA) = 20 / 70 to 25 / 75 (10 min) (A and B), MeCN (0.1% TFA) / H_2O (0.1% TFA) = 20 / 80 (C, D, E, F, G, and H), MeCN (0.1% TFA) / H_2O (0.1% TFA) = 17 / 83 (I and J), respectively.

^{18}F-labeling reaction for RO-948

[^{18}F]RO-948 was synthesized by a method slightly modified from a previous report.[3] Kryptofix222 (10.0 mg) was added to the reaction vessel with [^{18}F]fluoride, and the mixture was evaporated azeotropically with dry acetonitrile (300 μL) under a nitrogen gas stream at 120 °C, three times. Nitro precursor (0.4 mg) dissolved in dry DMSO (500 μL) was added to the reaction vessel. The mixture was microwave irradiated at 50 W for 240 s (PETwave, CEM, North Carolina, U.S.A.). After passing through the Cosmonice Filter (S) (0.45 μm, 4 mm) (Nacalai Tesque), the crude ^{18}F-labeled probes were purified by HPLC on a Cosmosil 5C_{18}-MS-II column with a gradient solvent of acetonitrile/H_2O/Et_3N at a flow rate of 1.0 mL/min.

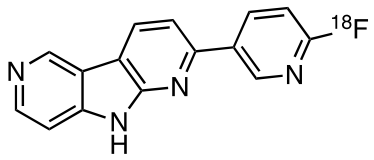
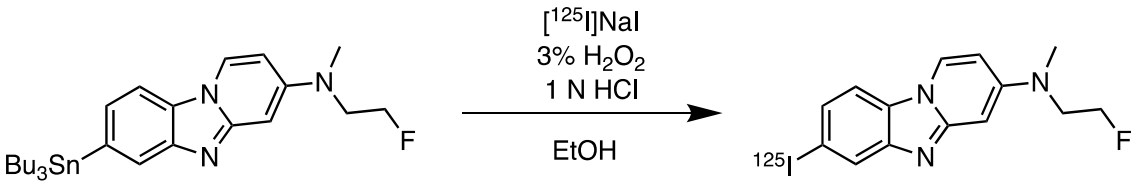


Figure S2. Chemical structure of [^{18}F]RO-948

^{125}I-labeling reaction for IBIPF1

[^{125}I]IBIPF1 was synthesized by a conventional method.[4] The crude ^{125}I-labeled probe was purified by HPLC on a Cosmosil 5C_{18}-AR-II column with an isocratic solvent of acetonitrile/H_2O/TFA at a flow rate of 1.0 mL/min.



Scheme S1. Radiosynthesis of [^{125}I]IBIPF1.

Human brain tissue

Experiments using human subjects were performed in accordance with relevant guidelines and regulations and were approved by the Ethics Committee of Kyoto University. Informed consent was secured from all subjects in this study. Postmortem brain tissues from an autopsy-confirmed case of Alzheimer's disease were obtained from the Graduate School of Medicine, Kyoto University.

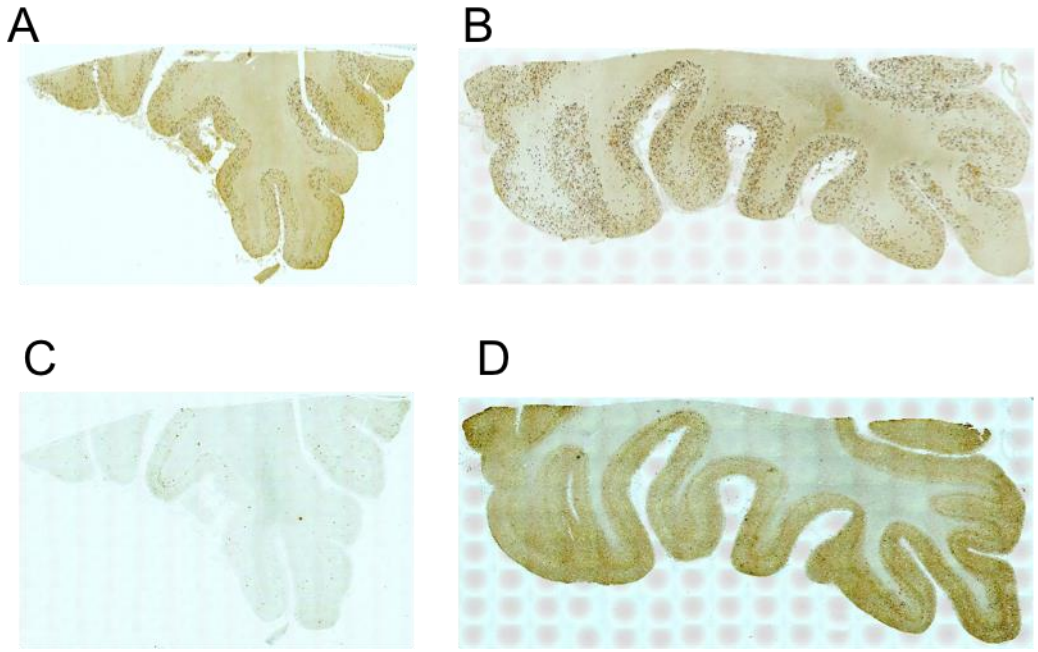


Figure S3. Immunohistochemical staining with antibodies against Aβ (A and B) and phosphorylated tau (C and D) of Alzheimer's disease brain sections. Adapted from reference.[1]

***In vitro* autoradiography with AD brain sections**

We performed this experiment according to the method reported previously.[2] The sections were incubated with ^{18}F-labeled probes (370 kBq/mL in 10% EtOH). The sections incubated with ^{18}F-labeled probes were washed with 50% EtOH for 3 min, twice. Autoradiographic images were obtained using an American Typhoon scanner system (GE Healthcare Life Sciences, Illinois, USA).

***In vitro* inhibition assay with AD brain sections**

The AD brain sections were incubated with the 10% EtOH solution containing [^{125}I]IBIPF1 (370 kBq/mL) and each BIP derivative (0 or 100 nM). The sections were washed with 50% EtOH for 1 h and washed with water for 1 min. Autoradiographic images were obtained using an American Typhoon scanner system. After obtaining autoradiographic images, the region of interest (ROI) was set as Figure S4. Then, radioactivity accumulation in the white matter and gray matter was calculated based on the standard curve (Figure S5).

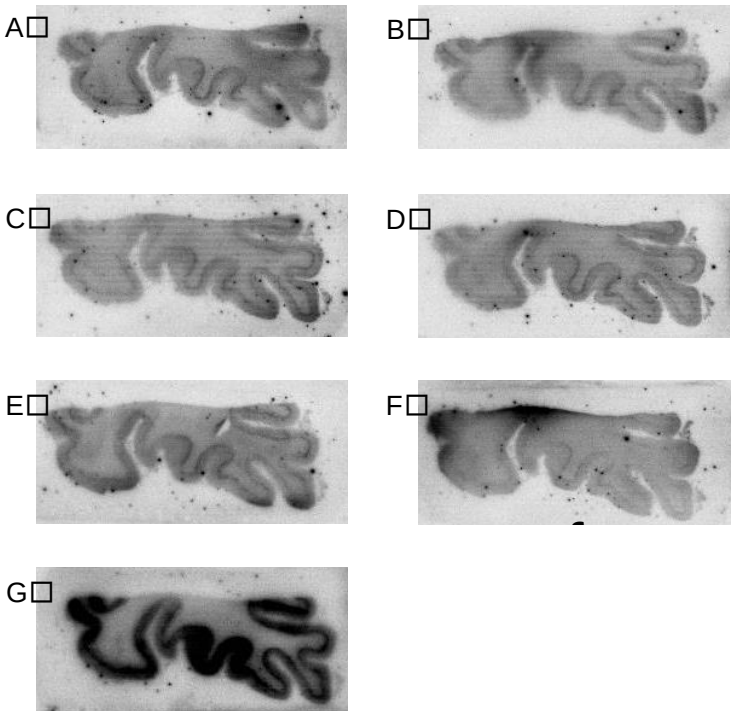


Figure S4. Comparison of *in vitro* autoradiography of [^{125}I]IBIPF1 in the presence of nonradioactive ligands (A: IBIPF, B: Br-BIPF, C: Cl-BIPF, D: Me-BIPF, E: OMe-BIPF, F: H-BIPF) and control (G: no non-radioactive ligand).

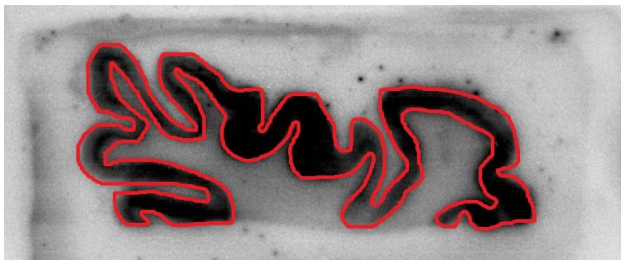


Figure S5. Regions of interest (ROI) used for calculation of the radioactivity accumulation in the gray matter.

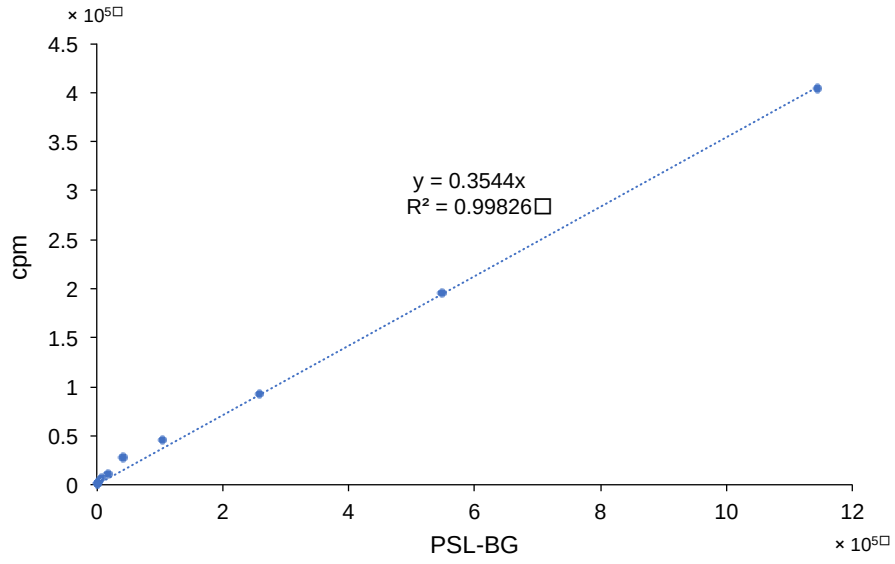


Figure S6. Standard curve used in *in vitro* inhibition assay with AD brain sections.

Animals

Animal experiments were conducted in accordance with our institutional guidelines and were approved by the Kyoto University Animal Care Committee. Male normal ddY mice were purchased from Japan SLC, Inc. (Shizuoka, Japan). ddY mice were fed standard chow and had free access to water.

***In vivo* biodistribution study using normal mice**

We conducted this study according to the method reported previously.[2] In this study, we injected ^{18}F-labeled probes (120 – 130 kBq/100 μL).

Table S2. Biodistribution of radioactivity after intravenous injection of [^{18}F]**1,** [^{18}F]**2,** [^{18}F]**3,** [^{18}F]**4,** [^{18}F]**5,** and [^{18}F]RO-948 in normal mice[a].

Tissue	Time after injection (min)			
	2	10	30	60
		[^{18}F]**1**		
Blood	3.82 (0.31)	2.97 (0.37)	3.08 (0.20)	3.45 (0.19)
Liver	13.7 (1.40)	18.1 (2.52)	11.4 (2.68)	5.59 (1.41)
Kidney	21.4 (2.40)	17.7 (2.42)	7.43 (0.34)	4.88 (2.52)
Intestine	4.99 (0.94)	10.2 (1.50)	14.8 (1.81)	16.9 (1.58)
Spleen	4.50 (0.70)	4.54 (0.23)	2.54 (0.22)	2.65 (0.40)
Pancreas	8.89 (1.60)	4.92 (0.79)	2.08 (0.18)	2.13 (0.40)
Heart	6.25 (0.97)	3.21 (0.33)	2.87 (0.14)	3.07 (0.55)
Lung	9.61 (1.67)	3.82 (0.37)	2.72 (0.15)	2.79 (0.16)
Stomach[b]	2.57 (0.31)	4.74 (0.81)	5.21 (1.09)	4.84 (0.81)
Brain	6.29 (0.91)	2.64 (0.15)	1.81 (0.16)	1.92 (0.22)
Bone	1.61 (0.40)	1.67 (0.43)	1.92 (0.28)	3.07 (1.17)
		[^{18}F]**2**		
Blood	3.74 (0.05)	3.87 (0.34)	4.08 (0.18)	4.13 (0.43)
Liver	7.69 (2.04)	9.05 (1.04)	4.94 (0.75)	3.60 (0.31)
Kidney	17.8 (3.38)	13.5 (0.79)	4.96 (0.92)	3.09 (0.34)
Intestine	4.41 (0.72)	9.63 (2.13)	12.7 (1.13)	13.6 (1.23)
Spleen	3.30 (0.61)	3.78 (0.42)	3.15 (0.25)	2.88 (0.44)
Pancreas	7.78 (1.10)	4.26 (0.74)	2.51 (0.23)	2.44 (0.25)
Heart	6.57 (0.41)	3.27 (0.50)	3.46 (0.19)	3.65 (0.40)

Lung	9.22 (0.85)	4.10 (0.35)	3.34 (0.24)	3.27 (0.34)
Stomach[b]	2.06 (0.38)	4.96 (2.33)	4.54 (0.71)	3.42 (0.65)
Brain	6.38 (0.55)	2.86 (0.22)	2.31 (0.19)	2.16 (0.22)
Bone	1.41 (0.38)	1.64 (0.62)	2.65 (0.23)	4.30 (0.84)
		[^{18}F]**3**		
Blood	5.26 (0.71)	4.64 (0.47)	4.08 (0.29)	4.14 (0.42)
Liver	12.3 (3.55)	12.8 (2.35)	13.7 (3.27)	10.9 (6.02)
Kidney	26.7 (4.76)	29.6 (7.22)	20.1 (6.60)	15.5 (6.33)
Intestine	6.22 (2.31)	8.61 (2.63)	16.5 (3.64)	19.3 (2.08)
Spleen	3.25 (0.84)	4.69 (0.19)	3.32 (0.39)	3.20 (0.67)
Pancreas	8.38 (1.86)	9.39 (0.40)	3.76 (0.59)	3.14 (1.16)
Heart	9.69 (5.49)	7.04 (3.88)	3.65 (0.42)	3.60 (4.50)
Lung	19.8 (5.29)	7.59 (1.64)	4.96 (0.34)	4.50 (0.80)
Stomach[b]	2.52 (0.59)	3.44 (1.25)	4.60 (1.02)	4.54 (1.48)
Brain	6.79 (0.83)	3.83 (0.51)	1.96 (0.17)	1.89 (0.11)
Bone	2.40 (0.53)	2.52 (0.49)	3.78 (1.30)	4.40 (0.86)
		[^{18}F]**4**		
Blood	2.86 (0.57)	2.61 (0.32)	2.95 (0.43)	3.16 (0.20
Liver	7.33 (0.90)	10.5 (1.36)	8.89 (2.48)	5.90 (0.82)
Kidney	20.1 (1.35)	27.3 (2.47)	23.1 (2.48)	13.2 (1.71)
Intestine	4.39 (0.73)	8.12 (0.85)	10.7 (1.26)	13.1 (2.23)
Spleen	3.50 (0.99)	5.23 (0.93)	4.51 (0.55)	4.01 (0.19)
Pancreas	6.36 (1.57)	6.46 (0.95)	3.66 (0.36)	2.97 (0.16)
Heart	10.3 (1.71)	4.66 (0.22)	3.56 (0.64)	3.98 (0.56)
Lung	16.7 (4.51)	6.87 (1.47)	4.22 (0.55)	3.98 (0.36)
Stomach[b]	2.10 (0.21)	3.08 (0.61)	4.69 (0.92)	4.60 (1.58)
Brain	5.15 (0.20)	3.01 (0.33)	1.88 (0.33)	1.83 (0.17)
Bone	2.60 (1.62)	2.26 (0.29)	2.99 (0.52)	4.13 (1.05)
		[^{18}F]**5**		
Blood	2.33 (0.18)	3.07 (0.26)	3.92 (0.51)	4.11 (0.34)
Liver	7.24 (0.44)	11.6 (2.03)	7.69 (1.68)	6.00 (0.84)
Kidney	25.1 (3.88)	28.8 (3.03)	11.4 (1.35)	4.94 (0.98)

Intestine	4.26 (0.33)	7.39 (0.26)	8.10 (0.79)	8.58 (0.77)
Spleen	3.65 (0.88)	4.71 (0.85)	3.35 (0.48)	3.07 (0.34)
Pancreas	7.80 (1.07)	7.41 (1.29)	3.05 (0.24)	2.36 (0.28)
Heart	7.71 (0.36)	4.03 (0.20)	3.65 (0.47)	3.81 (0.52)
Lung	10.0 (2.01)	4.07 (0.44)	1.99 (0.77)	3.70 (0.44)
Stomach[b]	2.21 (0.34)	4.22 (0.59)	4.88 (1.03)	4.07 (1.34)
Brain	4.84 (0.44)	2.87 (0.07)	1.99 (0.20)	2.02 (0.21)
Bone	1.19 (0.44)	2.19 (0.38)	3.46 (0.56)	3.89 (1.04)
		[^{18}F]RO-948		
Blood	1.94 (0.16)	2.16 (0.37)	1.29 (0.18)	0.51 (0.08)
Liver	8.17 (1.05)	18.1 (2.73)	10.4 (0.64)	5.61 (0.39)
Kidney	17.6 (4.48)	14.2 (1.19)	6.16 (0.90)	2.80 (0.37)
Intestine	3.79 (0.41)	7.96 (1.13)	11.0 (1.05)	13.4 (1.25)
Spleen	3.82 (1.34)	6.90 (1.20)	2.96 (0.90)	0.66 (0.25)
Pancreas	5.21 (0.98)	2.93 (0.27)	0.86 (0.04)	0.27 (0.11)
Heart	5.91 (0.48)	2.51 (0.23)	1.36 (0.08)	0.41 (0.09)
Lung	10.4 (1.62)	5.84 (0.84)	2.43 (0.23)	1.01 (0.16)
Stomach[b]	1.71 (0.28)	4.05 (0.64)	5.12 (0.99)	2.97 (0.69)
Brain	4.16 (0.35)	1.36 (0.17)	0.24 (0.08)	0.13 (0.07)
Bone	1.43 (0.61)	3.16 (0.57)	11.3 (2.27)	11.5 (2.49)

[a]Expressed as %ID/g. Each value represents the mean (SD) of 5 animals.

[b]Expressed as %ID.

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