Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

Supporting Information

Novel Radioiodinated 1,3,4-Oxadiazole Derivatives with Improved in Vivo Properties for SPECT Imaging of β-Amyloid Plaques

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Materials and methods

All reagents were commercial products and used without further purification unless otherwise indicated. ^{1}H NMR spectra were obtained at 400 MHz on JEOL JNM-AL400 NMR spectrometers at room temperature with TMS as an internal standard. Coupling constants are reported in hertz. Multiplicity is defined by s (singlet), d (doublet), t (triplet), and m (multiplet). High resolution mass spectra (HRMS) were obtained on JEOL JMS-700 MStation.

Chemistry

5-(5-(4-Bromophenyl)-1,3,4-oxadiazol-2-yl)-*N*,*N*-dimethylpyridin-2-amine (1).

To a mixture of 4-bromobenzhydrazide (215 mg, 1 mmol) and 6-(*N*,*N*-dimethylamino)nicotinic acid (166 mg, 1 mmol) was added $POCl_3$ (1.5 mL), and stirred at 85 ˚C for 3 h. Then, 1 M NaOH (5 mL) was added while the reaction mixture was cooled in an ice bath. After extraction of $CHCl_3$, the organic phase was dried over Na_2SO_4. The solvent was removed and residue was purified by silica gel chromatography ($CHCl_3$: MeOH = 9 : 1) to give 82 mg of **1** (24.8 %). TLC : R_f = 0.59

($CHCl_3$: MeOH = 9 : 1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.20 (s, 6H, $N(CH_3)_2$), 6.60 (d, J = 9.2 Hz, 1H, Pyridyl-H), 7.66 (d, J = 8.2 Hz, 2H, Phenyl-H), 7.97 (d, J = 8.2 Hz, 2H, Phenyl-H), 8.11 (dd, J = 2.3, 9.2 Hz, 1H, Pyridyl-H), 8.86 (d, J = 2.3 Hz, 1H, Pyridyl-H).

5-(5-(4-(Tributylstannyl)phenyl)-1,3,4-oxadiazol-2-yl)-*N*,*N*-dimethylpyridin-2-amine (2).

A mixture of **1** (53 mg, 0.16 mmol), bis(tributyltin) (0.3 mL), and $(Ph_3P)_4Pd$ (32 mg, 0.03 mmol) in a mixed solvent (6 mL, 1 : 1 = dioxane : Et_3N) was stirred at 90 ˚C for 2 h. The solvent was removed, and the resulting residue was purified by silica gel chromatography (ethyl acetate : hexane = 1 : 1) to give 24 mg of **2** (59.6 %). TLC : R_f = 0.56 (ethyl acetate : hexane = 1 : 1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88-0.95 (m, 9H, CH_3), 1.09-1.13 (m, 6H, CH_2), 1.30-1.54 (m, 6H, CH_2), 1.56-1.60 (m, 6H, CH_2), 3.19 (s, 6H, $N(CH_3)_2$), 6.60 (d, J = 8.7 Hz, 1H, Pyridyl-H), 7.62 (d, J = 7.3 Hz, 2H, Phenyl-H), 8.02 (d, J = 7.3 Hz, 2H, Phenyl-H), 8.14 (dd, J = 2.3, 8.9 Hz, 1H, Pyridyl-H), 8.88 (d, J = 2.3 Hz, 1H, Pyridyl-H).

5-(5-(4-Iodophenyl)-1,3,4-oxadiazol-2-yl)-*N*,*N*-dimethylpyridin-2-amine (3).

The same reaction as described above to prepare **1** was used, and 7 mg of **3** was obtained in 3.6 % yield from 4-iodobenzhydrazide and 6-(*N*,*N*-dimethylamino)nicotinic acid. TLC : R_f = 0.56 ($CHCl_3$: MeOH = 23 : 2).^{1}H NMR (400 MHz, $CDCl_3$) δ 3.20 (s, 6H, $N(CH_3)_2$), 6.60 (d, J = 8.7 Hz, 1H, Pyridyl-H), 7.81 – 7.89 (m, 4H, Phenyl-H), 8.12 (dd, J = 3.2, 8.7 Hz, 1H, Pyridyl-H), 8.86 (d, J = 2.3 Hz, 1H, Pyridyl-H). HRMS (FAB) $C_{15}H_{14}N_4OI$ found 393.0219, calcd m/z 393.0207(MH^+).

5-(5-(3-Bromophenyl)-1,3,4-oxadiazol-2-yl)-*N*,*N*-dimethylpyridin-2-amine (4).

The same reaction was used as described above to prepare **1**, and 57 mg of **4** was obtained in 34.4 % yield from 3-bromobenzhydrazide and 6-(*N*,*N*-dimethylamino)nicotinic acid. TLC : R_f = 0.50 ($CHCl_3$: MeOH = 49 : 1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.21 (s, 6H, $N(CH_3)_2$), 6.61 (d, J = 9.2 Hz, 1H, Pyridyl-H), 7.40 (t, J = 16.0 Hz, 1H, Phenyl-H), 7.66 (d, J = 10.0 Hz, 1H, Phenyl-H), 8.05 (d, J = 7.8 Hz, 1H, Phenyl-H), 8.13 (dd, J = 2.7, 8.9 Hz, 1H, Pyridyl-H), 8.25 (s, 1H,

Phenyl-H), 8.88 (d, *J* = 2.3 Hz, 1H, Pyridyl-H).

5-(5-(3-(Tributylstannyl)phenyl)-1,3,4-oxadiazol-2-yl)-*N*,*N*-dimethylpyridin-2-amine (5).

The same reaction was used as described above to prepare **2**, and 27 mg of **5** was obtained in 28.2 % yield from **4**. TLC : R_f = 0.57 (ethyl acetate : hexane = 1 : 2).^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88-1.61 (m, 27H), 3.20 (s, 6H, $N(CH_3)_2$), 6.61 (d, *J* = 9.6 Hz, 1H, Pyridyl-H), 7.46 (t, *J* = 7.3 Hz, 1H, Phenyl-H), 7.62 (d, *J* = 7.3 Hz, 1H, Phenyl-H), 8.01 (m, 1H, Phenyl-H), 8.16 (dd, *J* = 2.3, 9.2 Hz, 1H, Pyridyl-H), 8.18 (s, 1H, Phenyl-H), 8.88 (d, *J* = 2.3 Hz, 1H, Pyridyl-H).

5-(5-(3-Iodophenyl)-1,3,4-oxadiazol-2-yl)-*N*,*N*-dimethylpyridin-2-amine (6).

To a solution of **5** (27 mg, 0.05 mmol) in $CHCl_3$ (5 mL) was added a solution of iodine in $CHCl_3$ (1 mL, 0.25 M) at room temperature for 10 min, and saturated $NaHSO_3$ solution (2 mL) was added. The organic phase was separated, dried over Na_2SO_4, and purified by silica gel chromatography (ethyl acetate : hexane = 1 : 3) to give 17 mg of **6**

(89.2 %). TLC : R_f = 0.19 (ethyl acetate : hexane = 1 : 3). ^{1}H NMR (400 MHz, $CHCl_3$) δ 3.20 (s, 6H, $N(CH_3)_2$), 6.60 (d, J = 8.7 Hz, 1H, Pyridyl-H), 7.26 (t, J = 7.8 Hz, 1H, Phenyl-H), 7.86 (d, J = 6.9 Hz, 1H, Phenyl-H), 8.08 (d, J = 7.8 Hz, 1H, Phenyl-H), 8.15 (dd, J = 2.3, 9.2 Hz, 1H, Pyridyl-H), 8.45 (s, 1H, Phenyl-H), 8.88 (d, J = 2.3 Hz, 1H, Pyridyl-H). HRMS (FAB) $C_{15}H_{14}N_4OI$ found 393.0219, calcd m/z 393.0207(MH^+).

Radiolabeling

Radioiodinated forms of compounds **3** and **6** were prepared from the corresponding tributyltin derivatives by iododestannylation. Briefly, to initiate the reaction, 50 μL H_2O_2 (3%) was added to a mixture of a tributyltin derivative (50 μg/50 μL EtOH), [$^{123/125}$I]NaI (3.7 or 37 MBq), and 50 μL of 1 M HCl in a sealed vial. The reaction was allowed to proceed at room temperature for 10 min and terminated by the addition of $NaHSO_3$ (100 μL). After neutralization with sodium biocarbonate (100 μL) and extraction with ethyl acetate (1 mL), the extract was dried by passing through an anhydrous Na_2SO_4 column and then blown dry with a stream of nitrogen gas. The radioiodinated ligand was purified by HPLC on a Cosmosil C_{18} column (Nacalai Tesque,

Kyoto, Japan, $5C_{18}$-AR-II, 4.6 mm × 150 mm) with an isocratic solvent of H_2O/acetonitrile (4/6) at a flow rate of 1.0 mL/min.

Binding assays using the aggregated Aβ peptide in solution

A solid form of Aβ(1-42) was purchased from the Peptide Institute (Osaka, Japan). Aggregation was carried out by gently dissolving the peptide (0.25 mg/mL) in PBS (pH 7.4). The solution was incubated at 37 ˚C for 42 h with gentle and constant shaking. [^{125}I]IMPY (6-iodo-2-(4'-dimethylamino)phenyl-imidazo[1,2]pyridine) with 81.4 TBq specific activity and greater than 95 % radiochemical purity was prepared using the standard iodestannylation reaction as described previously [17]. Binding assays were carried out in 12 × 75 mm borosilicate glass tubes. A mixture containing 50 μL of [^{125}I]IMPY (0.02 nM diluted in 50% EtOH), 50 μL of Aβ(1–42) aggregates, and 850 μL of 10% EtOH was incubated at room temperature for 3 h. The mixture was then filtered through Whatman GF/B filters using a Brandel M-24 cell harvester, and the filters containing the bound ^{125}I ligand were placed in a gamma counter. Values for the half-maximal inhibitory concentration (IC_{50}) were determined from displacement curves

of six independent experiments using GraphPad Prism 4.0, and those for the inhibition constant (K_i) were calculated using the Cheng-Prus-off equation.

Determination of partition coefficient

Partition coefficients were measured by mixing [^{125}I]**3** and [^{125}I]**6** with 1.5 mL each of octanol and buffer (0.1 M phosphate, pH 7.4) in a test tube. The test tube was vortexed for 20 s three times. Two weighed samples (1 mL each) from the 1-octanol and buffer layers were measured for radioactivity with an automatic gamma counter (WIZARD^3 1480, PerkinElmer, MA, USA). The partition coefficient was determined by calculating the ratio of cpm/1 mL octanol to that of the buffer.

In vitro stability in mouse plasma

[^{125}I]**3** and [^{125}I]**6** (11 kBq, 10 μL) was added to the mouse plasma (200 μL), and the plasma samples were incubated at 37°C for 1 h. After incubation, plasma samples were mixed with equal volumes of acetonitrile followed by centrifugation at 4,500 rpm for 10 min to remove the denatured proteins. The supernatant was filtrated using 0.45 μm filter

(Millipore; Billerica, MA, USA). Then, the filtrate was analyzed by HPLC.

In vivo biodistribution in normal mice

Animal experiments were conducted in accordance with our institutional guidelines and approved by Kyoto University Animal Care Committee. A saline solution (100 μL) of radioiodinated agents containing EtOH (10 μL) was injected intravenously directly into the tail of ddY mice (5 weeks old, 24-26 g). The mice were sacrificed at various time points postinjection. The organs of interest were removed and weighed, and radioactivity was measured with an automatic gamma counter (WIZARD^3 1480, PerkinElmer).

Ex vivo autoradiography of transgenic mouse brain

Tg2576 transgenic mice (24 or 26 months old) and wild-type mice (18 or 24 months old) were purchased from Taconic Farms, Inc. (NY, USA). The animals were sacrificed at 70 min postinjection. Brains were immediately removed and frozen in a dry ice/hexane bath. Sections of 20 μm were cut and exposed to a BAS imaging plate

(Fuji Film, Tokyo, Japan) overnight. Ex vivo film autoradiograms were thus obtained. After autoradiographic examination, the same sections were stained by thioflavin S to confirm the presence of Aβ plaques. For thioflavin S staining, sections were immersed in thioflavin S solution (500 μM) containing 50% EtOH for 3 min and washed in 50% EtOH. After drying, the sections were examined using a microscope (BIOREVO BZ-9000, Keyence Corp., Osaka, Japan) equipped with a GFP-BP filter set (excitation, 450-490 nm; diachronic mirror, 495 nm; barrier filter, 510-560 nm).

SPECT study

SPECT images were obtained using the U-SPECT-Ⅱ system (MILabs, Utrecht, the Netherlands). A *Tg2576* transgenic mouse (24 months, female) and a wild-type mouse (24 months, female) were used as an Alzheimer's model and an age-matched control, respectively. A dose of approximately 44-48 MBq of [^{123}I]**6** in 10% aqueous EtOH (150 μL) was injected into the tail vein. After 10 min injection, the animals were anesthetized with 2.5% isoflurane. When fully anesthetized, the animal was placed on the scanner bed, with a nose cone used to maintain anesthesia at 2% isoflurane throughout the study.

The SPECT scans were acquired over 20-80 min after injection. The SPECT data were reconstructed by the OSEM method.

In vitro autoradiography using human AD brains

Postmortem brain tissues from an autopsy-confirmed case of AD (78 years old, female) were obtained from the Graduate School of Medicine, Kyoto University. Experiments were performed according to the regulations of the ethics committee of Kyoto University. Paraffin sections were subjected to two 10-min incubations in xylene, two 1-min incubations in 100% EtOH, one 1-min incubation in 90% EtOH, one 1-min incubation in 80% EtOH, and 1-min incubation in 70% EtOH for completely deparaffinization, followed by 30 s washes in water. The sections were incubated with [^{125}I]**3** and [^{125}I]**6** (148 kBq) for 1 h at room temperature. They then underwent two 1-min washes in 50% EtOH. After drying, ^{125}I-labeled sections were exposed to a BAS imaging plate (Fuji Film) for 24 h. The presence and localization of plaques on the sections were confirmed with immunohistochemical staining using a monoclonal Aβ antibody BC05 (Wako, Osaka, Japan).

Table S1. Biodistribution of radioactivity after injection of [^{125}I]**3** and [^{125}I]**6** in normal mice[a].

Tissue	Time after injection (min)			
	2	10	30	60
		[^{125}I]**3**		
Blood	1.93 (0.30)	2.33 (0.24)	1.97 (0.26)	1.79 (0.34)
Liver	6.41 (1.03)	7.44 (1.21)	3.86 (0.73)	2.61 (0.49)
Kidney	6.43 (0.74)	4.61 (0.58)	2.96 (0.46)	2.99 (1.28)
Intestine	1.50 (0.32)	3.38 (0.67)	5.29 (0.55)	8.57 (1.55)
Spleen	2.97 (0.36)	3.01 (0.99)	1.72 (0.24)	1.74 (0.48)
Pancreas	4.92 (0.56)	3.07 (0.31)	1.67 (0.29)	1.27 (0.19)
Heart	4.48 (0.43)	2.15 (0.43)	1.31 (0.16)	1.13 (0.19)
Stomach[b]	0.91 (0.33)	2.49 (0.58)	7.25 (2.17)	10.24 (2.64)
Brain	3.88 (0.54)	3.03 (0.39)	1.05 (0.18)	0.40 (0.05)
		[^{125}I]**6**		
Blood	1.82 (0.17)	1.65 (0.15)	1.67 (0.06)	1.76 (0.18)
Liver	6.06 (3.51)	11.50 (1.10)	8.42 (1.11)	5.94 (1.25)
Kidney	8.85 (0.73)	4.59 (0.53)	2.95 (0.20)	2.30 (0.44)
Intestine	2.28 (0.25)	4.08 (0.55)	8.77 (2.56)	12.08 (2.68)
Spleen	2.67 (0.61)	1.74 (0.19)	1.39 (0.27)	1.21 (0.12)
Pancreas	5.15 (0.76)	2.46 (0.25)	1.56 (0.09)	1.28 (0.18)

Heart	5.76 (1.04)	2.39 (0.17)	1.27 (0.31)	1.14 (0.20)
Stomach[b]	0.86 (0.10)	2.01 (0.21)	5.67 (0.57)	7.90 (1.65)
Brain	4.12 (0.47)	2.63 (0.23)	0.87 (0.06)	0.48 (0.14)

[a]Expressed as % injection dose per gram. Each value is the mean (SD) for 5 animals.

[b]Expressed as % injected dose per organ.

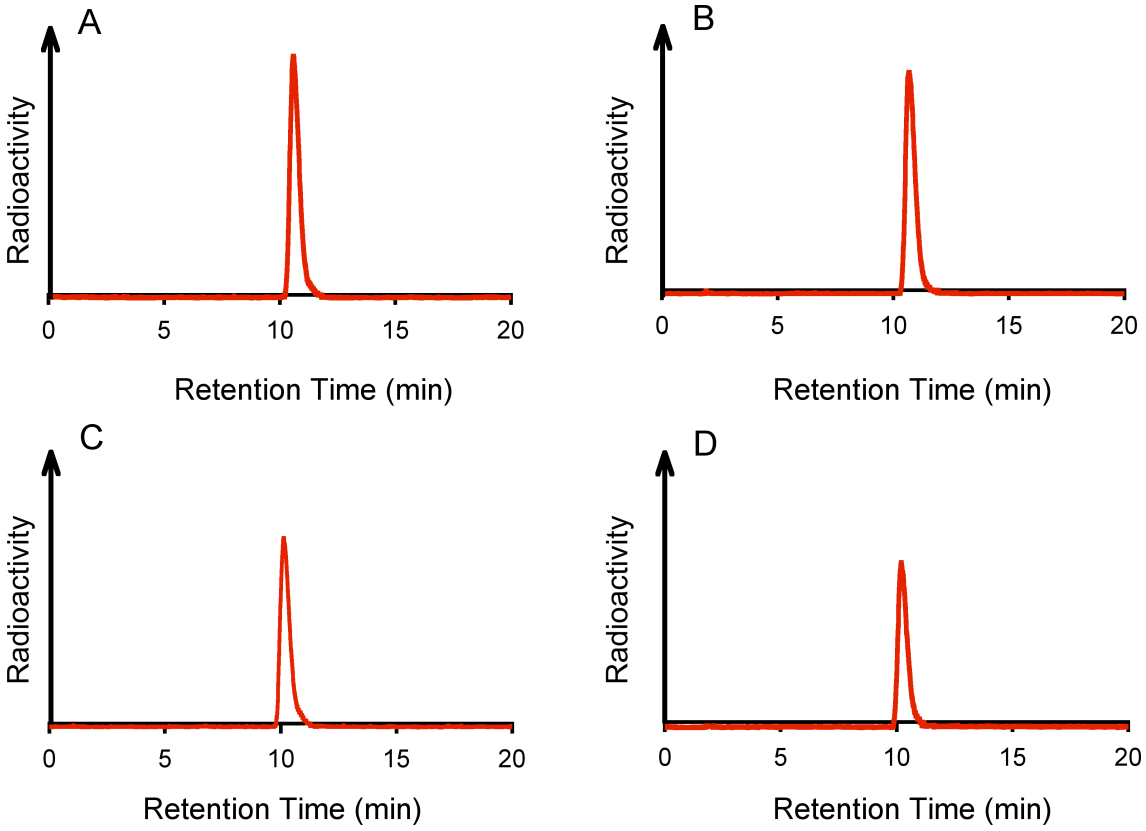

Figure S1. HPLC profiles of [^{125}I]**3** and [^{125}I]**6** in mouse plasma before (A and C) and after incubation for 1h (B and D) at 37°C.

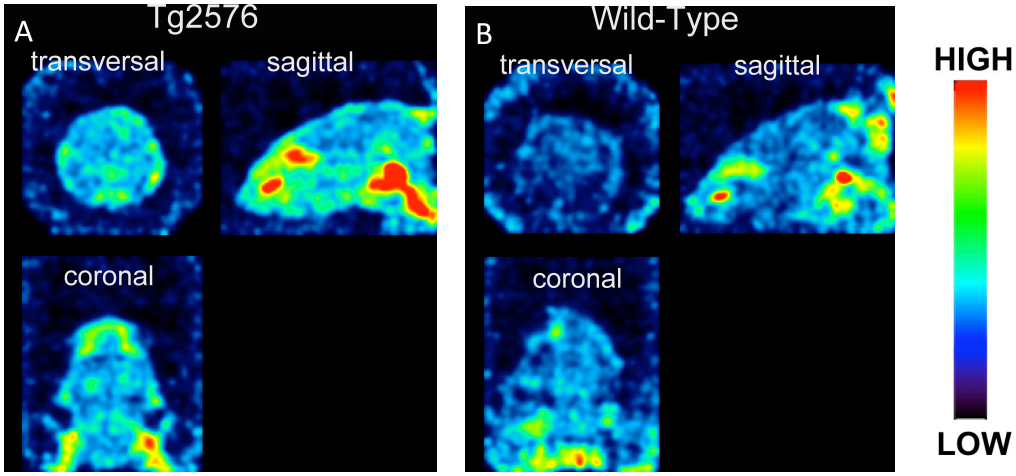

Figure S2. Brain SPECT images of a *Tg2576* mouse (A) and a wild-type mouse (B) 20-80 min after injection of [^{123}I]**6**.