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^{99m}Tc/Re complexes based on flavone and aurone as SPECT probes for imaging cerebral β-amyloid plaques

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ABSTRACT

Two ^{99m}Tc/Re complexes based on flavone and aurone were tested as potential probes for imaging β-amyloid plaques using single photon emission computed tomography. Both ^{99m}Tc-labeled derivatives showed higher affinity for Aβ(1–42) aggregates than did ^{99m}Tc-BAT. In sections of brain tissue from an animal model of AD, the Re-flavone derivative **9** and Re-aurone derivative **19** intensely stained β-amyloid plaques. In biodistribution experiments using normal mice, ^{99m}Tc-labeled flavone and aurone displayed similar radioactivity pharmacokinetics. With additional modifications to improve their brain uptake, ^{99m}Tc complexes based on the flavone or aurone scaffold may serve as probes for imaging cerebral β-amyloid plaques.

Alzheimer's disease (AD) is a neurodegenerative disorder of the brain associated with irreversible cognitive decline, memory impairment, and behavioral changes. Currently, the only definitive confirmation of AD is by postmortem histopathological examination of β-amyloid plaques in the brain. The early appraisal of clinical symptoms for a diagnosis of AD is often difficult and unreliable. Numerous reports suggest the accelerated accumulation of β-amyloid plaques in the brain to be a key risk factor associated with AD. Consequently, the detection of individual β-amyloid plaques in vivo by single photon emission computed tomography (SPECT) or positron emission tomography (PET) should improve the diagnosis of and also accelerate the discovery of effective therapeutic agents for AD.[1–4] Many PET/SPECT probes for imaging β-amyloid based on Congo Red, thioflavin T, and DDNP have been reported. Among them, [^{11}C]PIB,[5,6] [^{11}C]SB-13,[7,8] [^{18}F]BAY94-9172,[9,10] [^{11}C]BF-227,[11] [^{18}F]FDDNP,[12,13] [^{123}I]IMPY,[14–16] and [^{18}F]AV-45[17,18] have been tested clinically and demonstrated potential utility. There are more SPECT scanners than PET imaging devices installed for routine clinical imaging, which provides a certain advantage to using SPECT imaging agents. Since SPECT is more valuable than PET in terms of routine diagnostic use, the development of more useful Aβ imaging agents for SPECT has been a critical issue. Although many radioiodinated SPECT imaging agents for detecting β-amyloid plaques have been reported, there are few reports on the development of ^{99m}Tc imaging agents.

^{99m}Tc ($T_{1/2}$ = 6.01 h, 141 keV) has become the most commonly used radionuclide in diagnostic nuclear medicine by SPECT for several reasons: it is readily produced by an ^{99}Mo/^{99m}Tc generator, the gamma-ray energy it emits is suitable for detection, and its physical half-life is compatible with the biological localization and residence time required for imaging. Its ready availability, essentially 24 h a day, and easiness of use make it the radionuclide of choice. New ^{99m}Tc-labeled imaging agents will provide simple, convenient, and widespread SPECT-based imaging methods for detecting and eventually quantifying β-amyloid plaques in living brain tissue.

It has been reported that a dopamine transporter imaging agent, [^{99m}Tc]TRODAT-1, is useful to detect the loss of dopamine neurons in the basal ganglia associated with Parkinson's disease. This is the first example of a ^{99m}Tc imaging agent that can penetrate the blood–brain barrier via a simple diffusion mechanism and localize at sites in the central nervous system. Based on this success, efforts were made to search for comparable ^{99m}Tc imaging agents that target binding sites on β-amyloid plaques in the brain of AD patients. Several ^{99m}Tc-labeled imaging probes have been developed (Fig. 1), but no clinical study of them has been reported.[19–22]

Recently, we have reported that flavonoids including chalcone, flavone, and aurone serve as useful molecular scaffolds in the development of imaging agents for β-amyloid plaques in the brain.[23–28] Initially, we designed and synthesized four ^{99m}Tc-labeled chalcone derivatives with monoamine-monoamide

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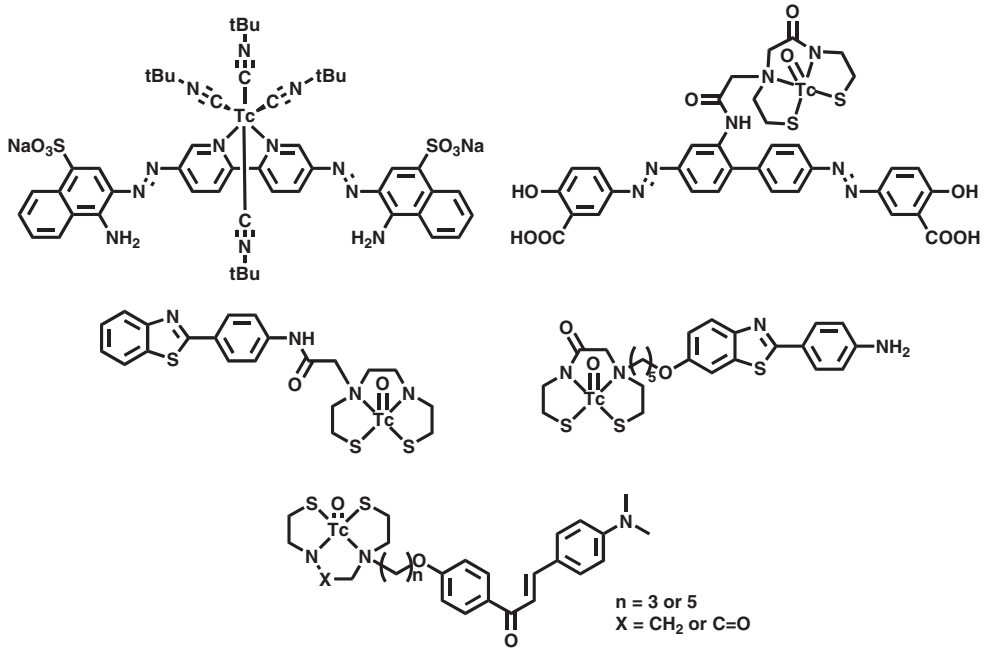
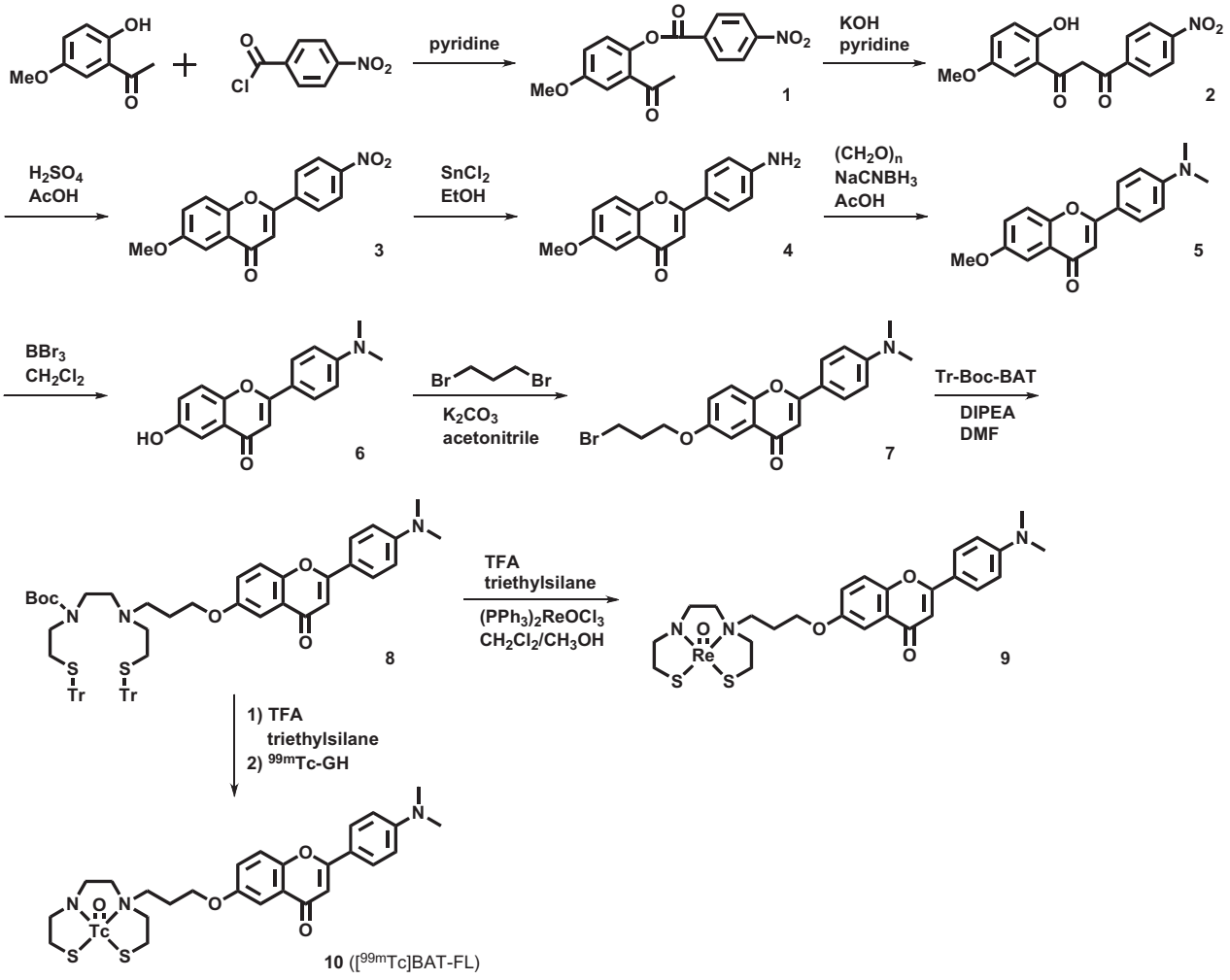
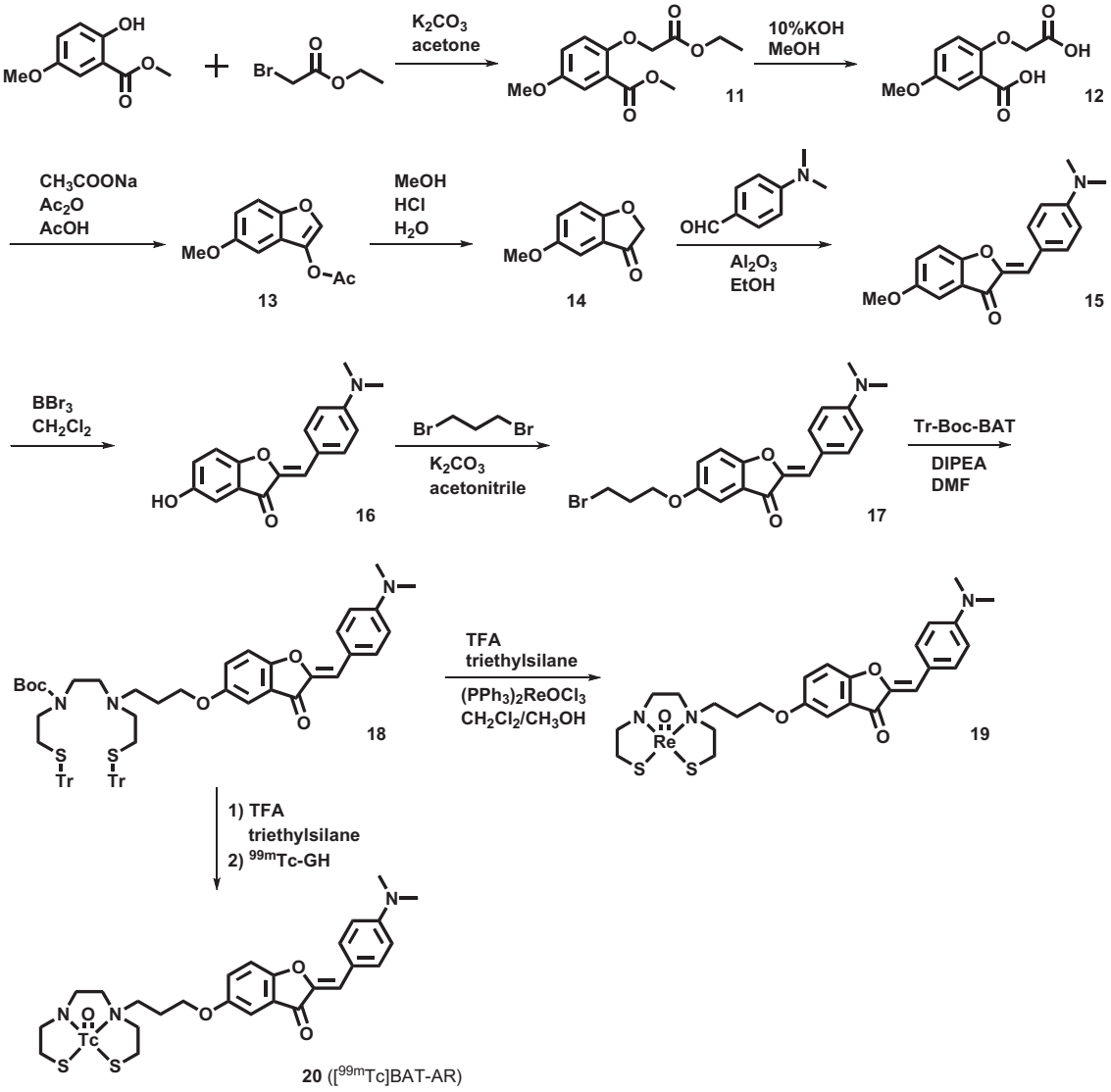


Figure 1. Chemical structure of ^{99m}Tc-labeled Aβ imaging probes reported previously.



Scheme 1. Synthesis of flavone derivatives.



Scheme 2. Synthesis of aurone derivatives.

dithiol (MAMA) and bis-amino-bis-thiol (BAT) (Fig. 1).[29] MAMA and BAT were selected as a chelation ligand taking into consideration the permeability of the blood–brain barrier, because they form an electrically neutral complex with ^{99m}Tc.[30] ^{99m}Tc-BAT-chalcone ($n = 3$) (Fig. 1) showed good uptake into and rapid clearance from the brain in addition to high affinity for β-amyloid plaques, indicating it may be a promising probe for the detection of β-amyloid plaques in the brain.[29] Based on the positive results, we decided to further develop new ^{99m}Tc imaging agents based on the flavonoid scaffold.

In the present study, to develop more useful ^{99m}Tc imaging agents for the clinical diagnosis of AD, we synthesized two flavone and aurone derivatives with BAT as a chelation ligand. We then evaluated the biological potential of these compounds as probes by testing their affinity for Aβ aggregates and β-amyloid plaques in sections of brain tissue from Tg2576 mice and their uptake by and clearance from the brain in biodistribution experiments using normal mice. Also, we compared their usefulness as Aβ imaging probes with a ^{99m}Tc-labeled chalcone derivative reported previously.[29] To our knowledge, this is the first time ^{99m}Tc/Re complexes based on flavone and aurone scaffolds have been proposed as probes for the detection of β-amyloid plaques in the brain.

The synthesis of the ^{99m}Tc/Re complexes based on flavone and aurone was outlined in Schemes 1 and 2. The chelation ligand (BAT) was synthesized according to methods reported previously with some slight modifications.[30] The most useful method of preparing flavones is known as the Baker–Venkataraman transformation.[23] A hydroxyacetophenone was first converted into a benzoyl ester (**1**) which was then treated with a base, forming a 1,3-diketone (**2**). Treatment of this diketone with acid led to the generation of the desired flavone (**3**). The free amino derivative **4** was readily prepared from **3** by reduction with $SnCl_2$ (92% yield). Conversion of **4** to the dimethylamino derivative **5** was achieved by a method reported previously (83% yield). Compound **5** was converted to **6** by demethylation with BBr_3 in CH_2Cl_2 (40% yield). The reaction of dibromopentane with **6** produced the flavone derivative **7** with a trimethine group. Then, **7** was joined to Tr-Boc-BAT to generate **8** (the precursor of ^{99m}Tc/Re reaction). The target aurone derivatives were prepared as shown in Scheme 2. The synthesis of the aurone backbone was achieved via an Aldol reaction of benzofuranones with benzaldehydes using Al_2O_3. 5-Methozy-3-benzofuranone (**14**) was reacted with 4-dimethybenzaldehyde in the presence of Al_2O_3 in chloroform at room temperature to form **15** in a yield of 92%. The precursor of the reaction with ^{99m}Tc/Re, **18**, was obtained

Table 1
HPLC retention times of ^{99m}Tc/Re compounds and log *P* of ^{99m}Tc compounds

Re compounds	Retention time[a] (min)	^{99m}Tc compounds	Retention time[a] (min)	Log *P* of ^{99m}Tc compounds[b]
9	9.5	**10**	11.1	2.77 ± 0.04
19	14.6	**20**	16.6	2.23 ± 0.04

[a] Reversed-phase HPLC using a mixture of H_2O–acetonitrile (2:3) as a mobile phase.
[b] The measurement was done in triplicate and repeated three times. Each value represents the mean ± SD for three independent experiments.

as described for the synthesis of the flavone derivative **8**. After deprotection of the thiol groups in **8** and **18** in TFA and triethylsilane, the Re complexes (**9** and **19**) were prepared through a reaction with $(PPh_3)_2ReOCl_3$. The corresponding ^{99m}Tc complexes, **10** ([^{99m}Tc]BAT-FL) and **20** ([^{99m}Tc]BAT-AR), were prepared by a ligand exchange reaction employing the precursor ^{99m}Tc-glucoheptonate (GH). The resulting mixture was analyzed by reversed-phase HPLC, showing that a single radioactive complex formed with radiochemical purity higher than 95% after purification by HPLC. The identity of the complex was established by comparative HPLC using the corresponding Re complexes as a reference (Table 1). The retention times for [^{99m}Tc]BAT-FL and [^{99m}Tc]BAT-AR on HPLC (radioactivity) were 11.1 and 16.6 min, respectively. The retention times of the corresponding Re complexes on HPLC (UV detection) were 9.5 and 14.6 min, respectively.

In vitro binding experiments to evaluate the affinity of [^{99m}Tc]BAT-FL and [^{99m}Tc]BAT-AR for Aβ(1–42) aggregates were carried out in solutions. The percent radioactivity of [^{99m}Tc]BAT-FL and [^{99m}Tc]BAT-AR bound to aggregates increased dependent on the dose of Aβ(1–42), while [^{99m}Tc]BAT showed no marked affinity for the aggregates (Fig. 2). At all concentrations of Aβ aggregates, [^{99m}Tc]BAT-AR showed significantly greater affinity than [^{99m}Tc]BAT-FL. In these binding experiments, the non-specific binding of [^{99m}Tc]BAT-FL and [^{99m}Tc]BAT-AR was estimated at 1.62–1.85%. The affinity of [^{99m}Tc]BAT-FL and [^{99m}Tc]BAT-AR was less than that of ^{99m}Tc-labeled chalcone derivatives reported previously (Fig. 1).[29] The order in terms of strength of binding corresponded with that of radioiodinated flavonoids,[23–25] indicating that the scaffolds of the [^{99m}Tc]BAT complexes did not play an important role in the affinity for Aβ aggregates.

To confirm the affinity for β-amyloid plaques in the mouse brain, neuropathological fluorescent staining with Re derivatives (**9** and **19**) was carried out using Tg2576 mouse brain sections (Fig. 3). Many β-amyloid plaques were clearly stained with the derivatives (Fig. 3A and B), as reflected by the high affinity for Aβ aggregates in binding assays in vitro. The labeling pattern was consistent with that observed with thioflavin S (Fig.3C and D). These results suggest that [^{99m}Tc]BAT-FL and [^{99m}Tc]BAT-AR would bind to β-amyloid plaques in the mouse brain in addition to having affinity for synthetic Aβ(1–42) aggregates. Although [^{99m}Tc]BAT-AR showed greater affinity than [^{99m}Tc]BAT-AR in the binding assays in vitro, no marked difference in binding between [^{99m}Tc]BAT-FL and [^{99m}Tc]BAT-AR was observed in the fluorescent staining experiments.

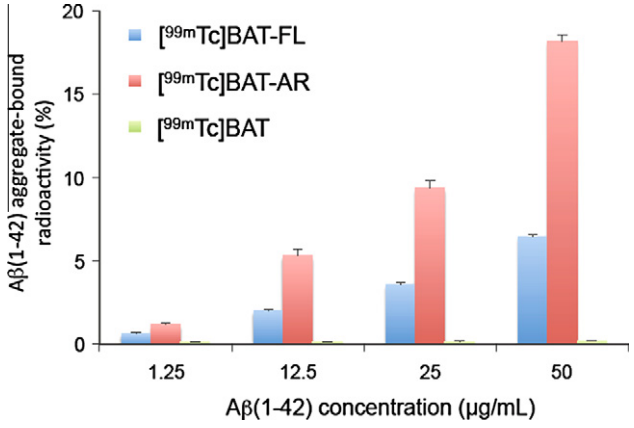


Figure 2. Binding assay of [^{99m}Tc]BAT-FL, [^{99m}Tc]BAT-AR, and [^{99m}Tc]BAT with Aβ(1–42) aggregates. Values are the mean ± standard error of the mean for three independent experiments.

[^{99m}Tc]BAT-FL and [^{99m}Tc]BAT-AR were examined as to their biodistribution in normal mice (Table 2). A biodistribution study provides important information on brain uptake. The ideal probe for imaging β-amyloid should penetrate the blood–brain barrier well enough to deliver a sufficient dose into the brain while clearing rapidly from normal regions so as to achieve a high signal to noise ratio in the AD brain. Previous studies suggest that the optimal lipophilicity for entry into the brain is obtained with log *P* values of between 1 and 3. [^{99m}Tc]BAT-FL and [^{99m}Tc]BAT-AR had log *P* values of 2.77 and 2.23, respectively (Table 1), but showed less uptake, 0.64 and 0.79%ID/g at 2 min postinjection, than expected. Thereafter, the radioactivity of [^{99m}Tc]BAT-FL and [^{99m}Tc]BAT-AR that accumulated in the brain was rapidly eliminated (0.23 and 0.11%ID/g at 60 min postinjection). Recently, we have reported that the ^{99m}Tc-labeled chalcone derivative showed high uptake (1.48%ID/g at 2 min postinjection) into and rapid clearance (0.17%ID/g at 60 min postinjection) from the brain, a highly desirable property for imaging agents for β-amyloid plaques.[29] The pharmacokinetics of the ^{99m}Tc-labeled chalcone derivative in the brain appears superior to that of any ^{99m}Tc-labeled probes reported previously, indicating that this compound should be investigated further as a potentially useful probe for imaging β-amyloid. Compared with that of the ^{99m}Tc-labeled chalcone,[29] the radioactivity of [^{99m}Tc]BAT-FL and [^{99m}Tc]BAT-AR appears insufficient for the imaging of β-amyloid plaques in the brain. Since the affinity of [^{99m}Tc]BAT-FL and [^{99m}Tc]BAT-AR for Aβ aggregates was as high as that of ^{99m}Tc-labeled chalcone derivatives,[29] improvement of the uptake of [^{99m}Tc]BAT-FL and [^{99m}Tc]BAT-AR is an important prerequisite to developing more useful ^{99m}Tc-labeled probes. Therefore, additional structural changes in the flavone and aurone scaffold are needed to further improve the pharmacokinetics of [^{99m}Tc]BAT-FL and [^{99m}Tc]BAT-AR in vivo.

In conclusion, we successfully designed and synthesized novel ^{99m}Tc/Re complexes based on flavone and aurone for the detection of β-amyloid plaques in the brain. Both [^{99m}Tc]BAT-FL and [^{99m}Tc]BAT-AR showed high affinity for synthetic Aβ(1–42) aggregates. In experiments in vitro using sections of brain from Tg2576 mice, Re complexes intensely stained β-amyloid plaques. In addition, [^{99m}Tc]BAT-FL and [^{99m}Tc]BAT-AR displayed good uptake into and a rapid washout from the brain after their injection in normal mice. This combination of affinity for β-amyloid plaques, and good uptake and clearance makes [^{99m}Tc]BAT-FL and [^{99m}Tc]BAT-AR promising probes for the detection of β-amyloid plaques in the brain, although additional modifications are required to enhance their uptake. The results of the present study should provide information useful for the development of ^{99m}Tc-labeled probes for the imaging of β-amyloid plaques in the brain.

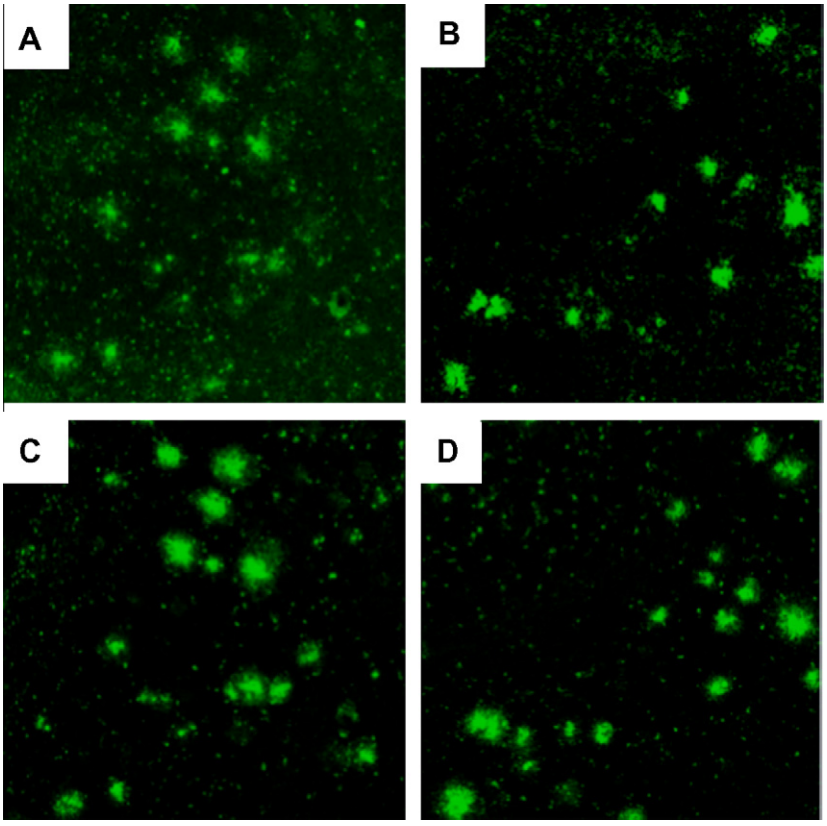


Figure 3. Fluorescent staining of the flavone derivative **9** (A), and aurone derivative **19** (B) in Tg2576 mouse brain. Labeled plaques were confirmed by staining the adjacent sections with thioflavin S (C and D).

Table 2
Biodistribution of radioactivity after injection of [^{99m}Tc]BAT-FL and [^{99m}Tc]BAT-AR in normal mice[a]

Organ	Time after injection (min)			
	2	10	30	60
[^{99m}Tc]BAT-FL (**10**)				
Blood	1.90 (0.08)	0.80 (0.16)	0.41 (0.06)	0.28 (0.06)
Liver	19.35 (1.30)	24.75 (3.45)	27.73 (3.30)	24.12 (3.08)
Kidney	9.70 (0.83)	5.56 (0.84)	2.38 (0.30)	1.40 (0.20)
Intestine[b]	4.54 (0.42)	11.36 (1.88)	26.61 (3.93)	42.67 (2.98)
Spleen	3.24 (0.61)	2.21 (0.31)	1.04 (0.42)	0.45 (0.07)
Lung	11.42 (2.10)	3.84 (0.57)	1.70 (0.24)	1.07 (0.16)
Stomach[b]	0.90 (0.15)	1.36 (0.55)	1.52 (0.67)	2.45 (1.04)
Pancreas	4.41 (0.29)	4.31 (0.35)	1.89 (0.15)	0.84 (0.17)
Heart	12.00 (1.16)	3.12 (0.51)	0.99 (0.18)	0.44 (0.09)
Brain	0.64 (0.07)	0.57 (0.14)	0.36 (0.01)	0.23 (0.04)
[^{99m}Tc]BAT-AR (**20**)				
Blood	1.56 (0.16)	0.71 (0.07)	0.35 (0.04)	0.21 (0.04)
Liver	17.76 (1.51)	17.77 (1.70)	15.17 (0.95)	12.96 (1.48)
Kidney	11.50 (0.73)	8.77 (1.15)	4.83 (0.77)	3.28 (1.52)
Intestine[b]	6.78 (0.78)	26.20 (2.45)	46.06 (3.17)	55.33 (7.42)
Spleen	2.87 (0.30)	1.92 (0.47)	0.70 (0.07)	0.35 (0.15)
Lung	6.10 (1.15)	3.25 (0.78)	1.63 (0.42)	0.85 (0.18)
Stomach[b]	1.03 (0.13)	1.63 (0.25)	1.88 (0.11)	1.69 (0.49)
Pancreas	5.85 (1.09)	4.20 (0.68)	1.53 (0.54)	0.60 (0.30)
Heart	12.30 (1.21)	3.26 (0.43)	1.15 (0.30)	0.40 (0.09)
Brain	0.79 (0.12)	0.70 (0.05)	0.27 (0.06)	0.11 (0.04)

[a] Each value represents the mean (SD) for 3–6 mice at each interval. Expressed as % injected dose per gram.
[b] Expressed as % injected dose per organ.

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Supplementary data

Supplementary data (procedure for the preparation of ^{99m}Tc/Re complexes, in vitro binding assay, in vitro fluorescent staining using Tg2576 mouse brain sections, and biodistribution experiments) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.08.004.

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