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Novel Benzofurans with ^{99m}Tc Complexes as Probes for Imaging Cerebral β-Amyloid Plaques

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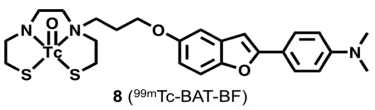
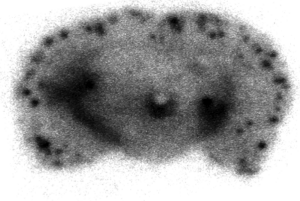
ABSTRACT Two novel benzofuran derivatives coupled with ^{99m}Tc complexes were tested as probes for imaging cerebral β-amyloid plaques using single photon emission tomography. Although both derivatives bound to Aβ(1−42) aggregates, ^{99m}Tc-BAT-BF showed higher affinity than ^{99m}Tc-MAMA-BF. In sections of brain tissue from an animal model of AD, ^{99m}Tc-BAT-BF clearly labeled β-amyloid plaques. In biodistribution experiments using normal mice, ^{99m}Tc-BAT-BF displayed high uptake soon after its injection and washed out from the brain rapidly, a highly desirable feature for an imaging agent. ^{99m}Tc-BAT-BF may be a potential probe for imaging β-amyloid plaques in Alzheimer's brains.

KEYWORDS Alzheimer's disease, β-amyloid plaque, Tc-99m, single photon emission computed tomography (SPECT), imaging



8 (^{99m}Tc-BAT-BF)



Alzheimer's disease (AD) is a neurodegenerative disease of the brain associated with irreversible cognitive decline, memory impairment, and behavioral changes. The presence of β-amyloid (Aβ) aggregates in the brain is generally accepted as a hallmark of AD.[1,2] Currently, the only definitive diagnosis of AD is by pathological examination of the postmortem staining of affected brain tissue, and the early appraisal of clinical symptoms for the diagnosis of AD is often difficult and unreliable. Thus, the detection of individual plaques in vivo by single photon emission tomography (SPECT) or positron emission tomography (PET) has been strongly desired to improve diagnosis and also accelerate the discovery of effective therapeutic agents for AD.[3−6] Many radiolabeled probes for imaging β-amyloid based on Congo Red, thioflavin T, and DDNP have been reported. Among them, [^{11}C]PIB,[7,8] [^{11}C]SB-13,[9,10] [^{18}F]BAY94-9172,[11,12] [^{11}C]BF-227,[13] [^{18}F]FDDNP,[14−16] [^{123}I]IMPY,[17−19] and [^{18}F]AV-45[20,21] have been tested clinically and demonstrated potential utility.

We have recently reported that ^{125}I-, ^{11}C-, and ^{18}F-labeled benzofuran derivatives showed high affinity for Aβ aggregates and good uptake into and rapid clearance from the brain, indicating that benzofuran can function as a promising scaffold for the development of β-amyloid imaging probes.[22,23] In this study, we planned the development of novel benzofuran derivatives labeled with technetium-99 m (^{99m}Tc). ^{99m}Tc ($T_{1/2}$ = 6.01 h, 141 keV) has become the most commonly used radionuclide in diagnostic nuclear medicine, because it is readily produced by an ^{99}Mo/^{99m}Tc generator; the medium γ-ray energy that 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. Several ^{99m}Tc-labeled imaging probes have been developed (Figure 1),[24−28] but no clinical study of them has been reported. 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.

In the present study, we synthesized two benzofuran derivatives with monoamine-monoamide dithiol (MAMA) and bis-amino-bis-thiol (BAT). 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.[29] We then evaluated their biological potential 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. To our knowledge, this is the first time that benzofurans coupled with ^{99m}Tc complexes have been proposed as probes for the detection of β-amyloid plaques in the brain.

The synthesis of the ^{99m}Tc/Re benzofuran derivatives is outlined in Scheme 1. The key step in the formation of the benzofuran backbone was readily achieved by reacting 2-hydroxy-5-methoxybenzaldehyde with 4-nitrobenzylbromide to produce compound **1** in a yield of 75%. The amino derivative **2** was prepared from **1** by reduction with $SnCl_2$ in a yield of 95%.

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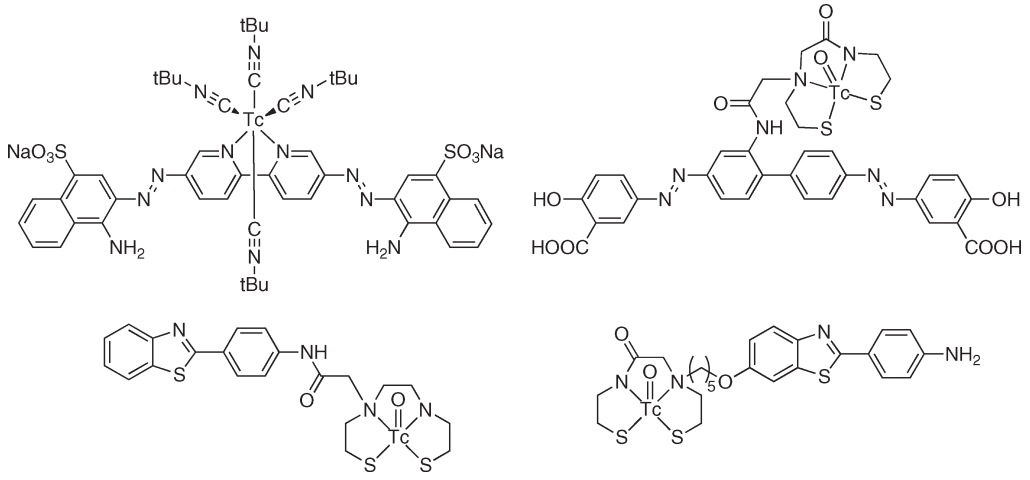
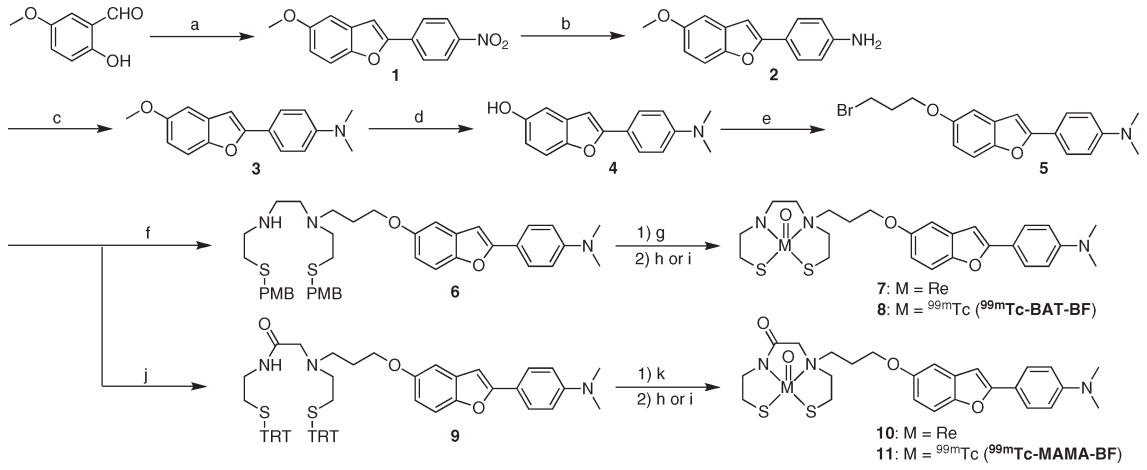


Figure 1. Chemical structure of ^{99m}Tc-labeled Aβ imaging probes reported previously.

Scheme 1. Synthesis of Re- and ^{99m}Tc-benzofuran Derivatives[a]



[a] Reagents: (a) DMF, K_2CO_3, 4-nitrobenzylbromide. (b) EtOH, $SnCl_2$. (c) CH_3COOH, $(CH_2O)_n$, $NaBH_3CN$. (d) CH_2Cl_2, BBr_3. (e) CH_3CN, K_2CO_3, 1,3-dibromopropane. (f) CH_3CN, DIPEA, PMB-BAT. (g) TFA, $MeSO_3H$, anisole. (h) CH_2Cl_2/MeOH, $(PPh_3)_2ReOCl_3$, AcONa. (i) CH_3CN, 0.1 N HCl, ^{99m}Tc-GH. (j) CH_3CN, TRT-MAMA, DIPEA. (k) TFA, Et_3SiH.

Conversion of **2** to the dimethylamino derivative **3** was achieved by an efficient method with paraformaldehyde, sodium cyanoborohydride, and acetic acid (78% yield). The *O*-methyl group of **3** was removed by reacting with BBr_3 to give **4** in a yield of 63%. After a trimethylene group was introduced into **4** as a linker by reacting with 1,3-dibromopropane, the chelation ligands were conjugated with **5**. The thiol-protected chelation ligands (PMB-BAT and TRT-MAMA) were synthesized according to methods reported previously with some slight modifications. Then, **5** was joined to PMB-BAT or TRT-MAMA to generate the compounds **6** (PMB-BAT-BF) and **9** (TRT-MAMA-BF), respectively. After deprotection of the thiol group in **6** and **9**, the Re complexes (**7** and **10**) were directly prepared by a reaction with $(PPh_3)_2ReOCl_3$. The corresponding ^{99m}Tc complex, **8** (^{99m}Tc-BAT-BF) or **11** (^{99m}Tc-MAMA-BF), was prepared by a ligand exchange reaction employing the precursor ^{99m}Tc-glucoheptonate (GH). The resulting mixture was analyzed by reversed-phase high-performance liquid chromatography (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. The retention times for ^{99m}Tc-BAT-BF and ^{99m}Tc-MAMA-BF on HPLC (radioactivity) were 13.2 and 10.3 min, respectively. The retention times of the corresponding Re complexes (**7** and **10**) on HPLC (UV detection) were 11.4 and 9.4 min, respectively.

To evaluate the binding affinity of Re-BAT-BF (**7**) and Re-MAMA-BF (**10**), inhibition assays with [^{125}I]IMPY and Aβ(1−42) aggregates were performed (Figure 2).[18] Both ligands inhibited the binding of [^{125}I]IMPY to Aβ(1−42) aggregates in a dose-dependent manner, indicating an affinity for Aβ aggregates. Their K_i values were 11.5 and 24.4 nM, respectively, suggesting that Re-BAT-BF displayed higher affinity than Re-MAMA-BF (Table 1). The results also indicated that ^{99m}Tc-BAT-BF and ^{99m}Tc-MAMA-BF would bind Aβ aggregates. Indeed, in subsequent assays, ^{99m}Tc-BAT-BF and ^{99m}Tc-MAMA-BF showed higher affinity than ^{99m}Tc-BAT and ^{99m}Tc-MAMA (Figure S1 in the Supporting Information). These results

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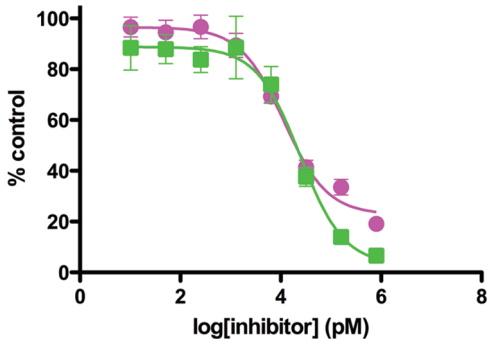

Figure 2. Inhibition curves of Re-BAT-BF (**7**) (pink circle) and Re-MAMA-BF (**10**) (green square) for the binding of [^{125}I]IMPY to Aβ(1−42) aggregates.

Table 1. Inhibition Constants for the Binding of [^{125}I]IMPY to Aβ(1−42) Aggregates

compound	K_i (nM)[a]
Re-BAT-BF (**7**)	11.5 ± 0.56
Re-MAMA-BF (**10**)	24.4 ± 0.77

[a] Values are the means ± standard errors of the mean of three independent determinations.

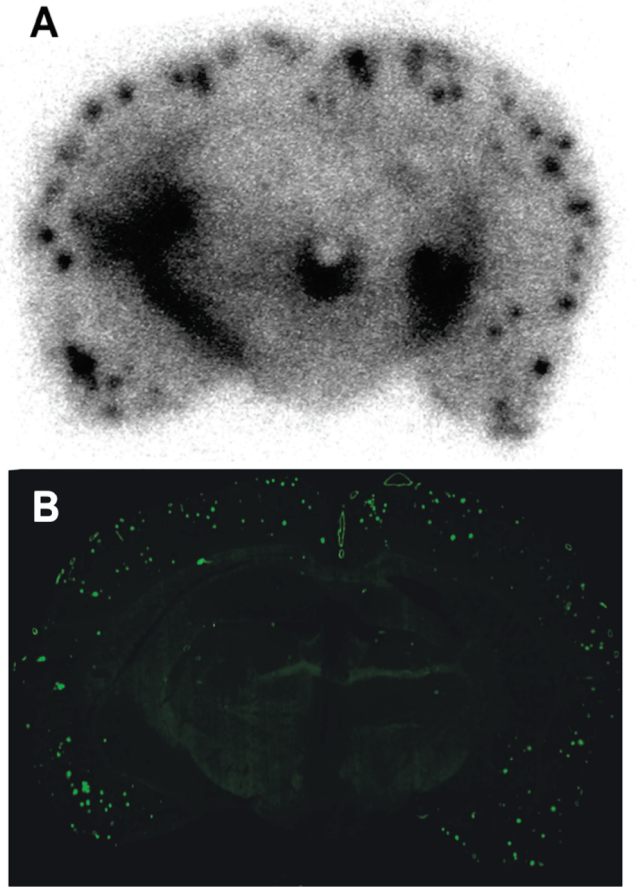

Figure 3. Autoradiography of ^{99m}Tc-BAT-BF in sections from Tg2576 mouse brain (A). Labeled plaques were confirmed by the staining of the adjacent sections with thioflavin-S (B).

strongly support our previous report that benzofuran derivatives have considerable tolerance for structural modifications.[22,23]

Next, the affinity of ^{99m}Tc-BAT-BF for β-amyloid plaques was investigated in vitro using sections of Tg2576 mouse brain (Figure 3). Autoradiographic images showed many radioactive spots in the brain tissue. Furthermore, the radioactivity of ^{99m}Tc-BAT-BF corresponded with the areas of staining with thioflavin-S, a pathological dye commonly used for β-amyloid plaques. In contrast, normal mouse brain displayed no remarkable accumulation of ^{99m}Tc-BAT-BF (data not shown). The results suggest that ^{99m}Tc-BAT-BF binds affinity for β-amyloid plaques in the mouse brain in addition to binding synthetic Aβ aggregates.

The biodistribution of ^{99m}Tc-BAT-BF and ^{99m}Tc-MAMA-BF was examined in normal mice (Table 2). A biodistribution experiment provides important information on uptake in the brain. The ideal imaging probe should penetrate the blood−brain barrier to deliver a sufficient dose into the brain but be rapidly cleared from normal regions so as to achieve in a high signal-to-noise ratio. ^{99m}Tc-BAT-BF showed greater uptake (1.34 % ID/g) than ^{99m}Tc-MAMA-BF (0.74 % ID/g) at 2 min postinjection. The uptake of ^{99m}Tc-BAT-BF peaked at 10 min postinjection, reaching 1.37 % ID/g, and about 60 % of radioactivity accumulated at 2 min postinjection had been washed out from the brain by 60 min. The uptake of

Table 2. Biodistribution of Radioactivity after Injection of ^{99m}Tc-Labeled Benzofuran Derivatives in Normal Mice[a]

	time after injection (min)			
organ	2	10	30	60
	^{99m}Tc-BAT-BF (**8**)			
blood	4.40 (0.27)	1.96 (0.06)	1.93 (0.26)	2.15 (0.91)
liver	21.94 (5.94)	20.87 (1.28)	19.65 (1.31)	15.09 (3.83)
kidney	10.28 (1.76)	7.90 (0.40)	4.27 (0.18)	2.70 (0.57)
intestine[b]	1.45 (0.18)	3.68 (0.52)	7.42 (1.62)	9.02 (1.93)
spleen	5.20 (1.01)	3.09 (0.23)	1.69 (0.21)	1.16 (0.14)
lung	26.70 (2.27)	6.48 (1.33)	3.51 (0.64)	2.36 (0.48)
stomach[b]	1.33 (0.57)	1.90 (0.43)	4.09 (1.37)	4.17 (1.92)
pancreas	4.14 (0.77)	4.57 (0.24)	2.98 (0.38)	1.42 (0.15)
heart	17.60 (2.60)	8.29 (0.97)	3.28 (1.35)	1.51 (0.25)
brain	1.34 (0.12)	1.37 (0.18)	0.94 (0.20)	0.56 (0.07)
	^{99m}Tc-MAMA-BF (**11**)			
blood	4.13 (0.42)	1.78 (0.25)	2.15 (0.12)	2.24 (0.24)
liver	20.17 (3.81)	21.62 (2.62)	23.32 (1.59)	20.16 (2.13)
kidney	7.37 (1.06)	8.09 (1.16)	5.11 (0.29)	3.28 (0.45)
intestine[b]	0.95 (0.22)	2.13 (0.19)	4.75 (0.93)	5.73 (0.66)
spleen	4.48 (0.56)	3.69 (0.34)	3.49 (0.61)	2.59 (0.65)
lung	24.04 (5.17)	7.59 (2.13)	4.24 (0.35)	3.54 (1.26)
stomach[b]	0.73 (0.21)	2.35 (0.58)	4.94 (0.57)	2.81 (0.51)
pancreas	2.70 (0.47)	4.00 (1.28)	5.48 (0.61)	3.76 (0.36)
heart	12.28 (2.20)	10.48 (1.79)	5.05 (0.90)	2.16 (0.34)
brain	0.74 (0.15)	0.99 (0.22)	1.23 (0.09)	0.89 (0.08)

[a] Each value represents the mean (SD) for five mice. Expressed as % injected dose per gram. [b] Expressed as % injected dose per organ.

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^{99m}Tc-MAMA-BF peaked 30 min after the injection at 1.23 % ID/g, and the washout from the brain was slower than that of ^{99m}Tc-BAT-BF throughout the time course, which is unsuitable for imaging in vivo. The log *P* values of ^{99m}Tc-BAT-BF and ^{99m}Tc-MAMA-BF were 3.33 and 3.01, respectively. Although lipophilicity is just one of the factors affecting the uptake of a compound into the brain,[4] it may explain the good uptake of ^{99m}Tc-BAT-BF.

In conclusion, we successfully designed and synthesized novel benzofuran derivatives conjugated with ^{99m}Tc or Re complexes for the detection of β-amyloid plaques in the brain. In experiments in vitro, Re-BAT-BF bound to Aβ aggregates with greater affinity than did Re-MAMA-BF, and ^{99m}Tc-BAT-BF clearly labeled β-amyloid plaques in sections of brain tissue from Tg2576 mice. In addition, ^{99m}Tc-BAT-BF displayed good uptake into and a rapid washout from the brain after its injection in normal mice. The combination of good affinity for β-amyloid plaques, uptake, and clearance makes ^{99m}Tc-BAT-BF a promising probe for the detection of β-amyloid plaques in the brain. The results of the present study should provide useful information for the development of ^{99m}Tc-labeled probes for the imaging of β-amyloid plaques in the brain.

SUPPORTING INFORMATION AVAILABLE Procedures for the preparation of new ligands, analysis of data, experiments in vitro, and biodistribution experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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