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Synthesis and biological evaluation of novel technetium-99m labeled phenylbenzoxazole derivatives as potential imaging probes for β-amyloid plaques in brain

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ABSTRACT

Two uncharged ^{99m}Tc-labeled phenylbenzoxazole derivatives were biologically evaluated as potential imaging probes for β-amyloid plaques. The ^{99m}Tc and corresponding rhenium complexes were synthesized by coupling monoamine–monoamide dithiol (MAMA) and bis(aminoethanethiol) (BAT) chelating ligand via a pentyloxy spacer to phenylbenzoxazole. The fluorescent rhenium complexes **6** and **9** selectively stainined the β-amyloid plaques on the sections of transgenic mouse, and showed high affinity for $A\beta_{(1-42)}$ aggregates (K_i = 11.1 nM and 14.3 nM, respectively). Autoradiography in vitro indicated that [^{99m}Tc]**6** clearly labeled β-amyloid plaques on the sections of transgenic mouse. Biodistribution experiments in normal mice revealed that [^{99m}Tc]**6** displayed moderate initial brain uptake (0.81% ID/g at 2 min), and quickly washed out from the brain (0.25% ID/g at 60 min). The preliminary results indicate that the properties of [^{99m}Tc]**6** are promising, although additional refinements are needed to improve the ability to cross the blood–brain barrier.

Alzheimer's disease (AD) is the leading cause of neurodegenerative disorder of the brain, accounting for most dementia cases after the age of 60. Histopathologically, the formation of extracellular β-amyloid (Aβ) plaques is one of the pathological hallmarks of this disease.[1] In clinic, it is very difficult to differentiate AD from other dementia cases. Currently, diagnosis of AD relies on the results from neuropsychological tests. However, this method is often complicated and unreliable, where the degree of accuracy ranges from 50% to 90%. A definite diagnosis of AD can only be confirmed by histopathological observation of Aβ plaques in the cerebral cortex of postmortem brain tissue. At present, the exact etiology of AD is not completely understood, the most widely accepted theory regarding to this disease is the amyloid cascade hypothesis.[2–4] Therefore, Aβ represents an important molecular target for AD, and the detection of deposited Aβ plaques with non-invasive techniques such as positron emission tomography (PET) or single photon emission computed tomography (SPECT) could achieve the differential diagnosis of AD in its pre-symptomatic stages.[5,6]

Over the past decade, based on the scaffolds of thioflavin-T (ThT) and Congo Red (CR), (commonly used dyes for detection of Aβ plaques), several specific radiotracers were synthesized and evaluated as in vivo imaging probes for Aβ plaques with PET and SPECT. Some PET tracers such as [^{11}C]PIB,[7,8] [^{18}F]FDDNP,[9–11] [^{11}C]SB-13,[12] [^{18}F]BAY94-9172,[13] [^{18}F]GE-067[14] and [^{18}F]AV-45[15,16] have been evaluated in clinical trials.

However, the development of SPECT tracers was lagging far behind, to the best of our knowledge, a radioiodinated ligand [^{123}I]IMPY, is the only SPECT tracer tested in humans. Some undesirable properties including high lipophilicity, in vivo instability together with insufficient target-to-background ratio may account for the failure of [^{123}I]IMPY in clinical trials.[17–19] Furthermore, some other radioiodinated ThT analogs for SPECT such as TZDM,[20] IBOX,[21] phenylbenzofuran derivatives[22] and phenylindole derivatives[23] have shown high affinity for Aβ aggregates in vitro and high initial uptake, but their potential use was limited by the slow washout rate from the brain and the lack of routine availability of ^{123}I radioisotope.

At present, the most widely used radionuclide in diagnostic nuclear medicine for SPECT imaging is technetium-99m (^{99m}Tc), mainly due to its optimal nuclear properties ($T_{1/2}$ = 6.01 h, 141 keV), easy availability (through commercial ^{99}Mo/^{99m}Tc generators), and low cost. Accordingly, the development of ^{99m}Tc-labeled imaging probes targeting Aβ plaques will provide simple, convenient, and widespread method for the diagnosis of AD. In the past few years, several ^{99m}Tc-labeled imaging probes have been developed (Fig. 1). Such as the initially reported ^{99m}Tc-labeled Congo Red[24,25] and Chrysamine G derivatives,[26,27] which displayed high affinity for Aβ aggregates in vitro. However, due to the large molecular weight and ionized character at physiological pH, they cannot cross the blood–brain barrier (BBB). Attempts to prepare

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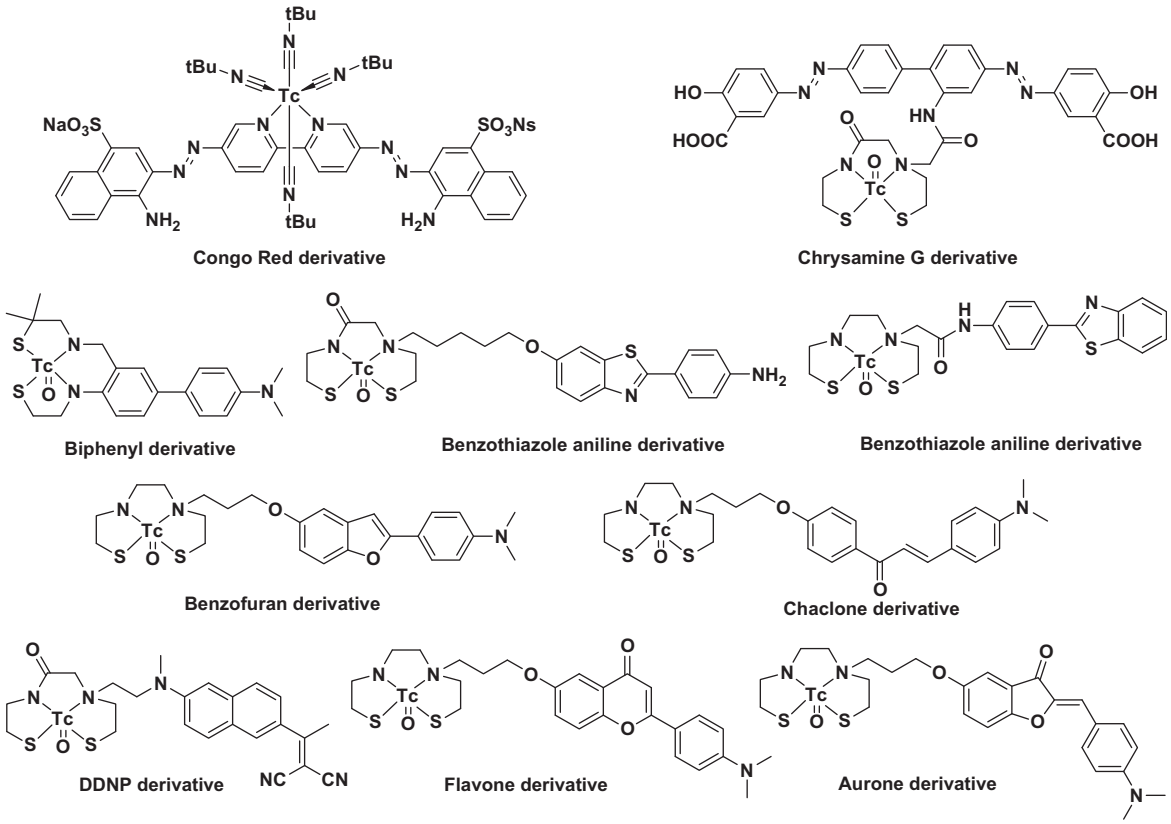

Figure 1. Chemical structures of ^{99m}Tc-labeled Aβ imaging probes reported previously.

^{99m}Tc-labeled nonpolar, neutral, small in size and more lipophilic ligands have been reported. Kung and his co-workers reported the ^{99m}Tc-labeled N_2S_2-biphenyl derivatives, which showed high initial brain uptake (1.18% dose/organ at 2 min), but it failed to bind to the Aβ plaques in the postmortem human brain tissue of patients with confirmed AD.[28] Some other groups reported a series of benzothiazole aniline (BTA),[29–31] phenylbenzofuran,[32] DDNP,[33] chaclone,[34] flavone and aurone[35] derivatives conjugated with bis(aminoethanethiol) (BAT) or monoamine–monoamide dithiol (MAMA) ligands. Although it was demonstrated that these ^{99m}Tc-labeled derivatives bind to Aβ plaques in vitro, they still cannot cross BBB to a sufficient degree for the in vivo imaging.

Recently, we have synthesized and evaluated a series of ^{18}F-labeled phenylbenzoxazole derivatives as novel Aβ imaging probes. They showed high affinity for Aβ aggregates and favorable pharmacokinetics in vivo, which suggests that this class of tracers has potential applications for PET imaging.[36] Building on the previous results, we attempt to design novel ^{99m}Tc-labeled phenylbenzoxazole derivatives for SPECT imaging. In the present study, we report the synthesis of two phenylbenzoxazole derivatives conjugated to MAMA and BAT via a pentyloxy spacer as well as their complexation to Re and ^{99m}Tc. We also report the biological evaluation of these two probes as novel Aβ imaging probes.

The synthetic route of the phenylbenzoxazole derivatives is outlined in Scheme 1. The key step in the formation of the phenylbenzoxazole backbone was achieved by reacting 2-amino-4-methoxyphenol with 4-(dimethylamino)benzoic acid in PPA, compound **1** was obtained in a yield of 39.6%. Subsequent demethylation of the methoxyl group using BBr_3 in CH_2Cl_2 afforded **2** in a yield of 89.5%. The thiol-protected chelation ligands (PMB-MAMA and *N*-Boc-Tr-BAT) were synthesized according to the methods reported previously.[37] After introduction of the pentyloxy linker into **2** by reacting with 1,5-dibromo pentane, **3** were conjugated to PMB-MAMA or *N*-Boc-Tr-BAT to generate the compounds **4** and **7**, respectively. Compounds **5** and **8** (the precursors for ^{99m}Tc labeling) were obtained by deprotection of the thiol groups in **4** and **7**. The rhenium complexes (**6** and **9**) were prepared by the reaction of **4** and **7** with $(PPh_3)_2ReOCl_3$ in CH_2Cl_2. The corresponding ^{99m}Tc complexes [^{99m}Tc]**6** and [^{99m}Tc]**9** were prepared by a ligand exchange reaction employing the precursor ^{99m}Tc-glucoheptonate (GH) in boiling water, and the desired products were formed as major radioactive products. After purification by reversed-phase high-performance liquid chromatography (HPLC), the final products were obtained with radiochemical purity higher than 95%. The identity of the complexes were established by comparative HPLC using the corresponding Re complexes as reference. The retention time of [^{99m}Tc]**6** and [^{99m}Tc]**9** on HPLC (radioactivity) were 7.92 and 9.97 min, respectively. The retention times of the corresponding rhenium complexes (**6** and **9**) on HPLC (UV detection) were 7.46 and 9.31 min, respectively (Fig. S1 in the Supplementary data).

The specific binding of the rhenium complexes **6** and **9** to Aβ plaques were evaluated by neuropathological staining with the brain sections from a transgenic mouse (C57BL6, APPswe/PSEN1, 12 months old), an animal model for AD. As shown in Figure 2A and C, both Re complexes could clearly stain Aβ plaques on the brain sections with low background, and the similar stain pattern of Aβ plaques was consistent with that stained with thioflavin-S using the adjacent sections (Fig. 2B and D). The results indicated that these two Re complexes could bind specifically to Aβ plaques. Furthermore, the quantitative binding affinities of rhenium complexes **6** and **9** for $A\beta_{1-42}$ aggregates were determined by competition binding assay using [^{125}I]IMPY as radio-ligand, while IMPY was also screened using the same system for comparison. As shown in Table 1, complexes **6** and **9** displayed high affinity to $A\beta_{1-42}$ aggregates (K_i = 11.1 nM and 14.3 nM, respectively), which