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Synthesis and biological evaluation of ^{99m}Tc, Re-monoamine-monoamide conjugated to 2-(4-aminophenyl) benzothiazole as potential probes for β-amyloid plaques in the brain

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Abstract—The benzothiazole aniline (BTA) conjugated with monoamine-monoamide (MAMA) was synthesized and then labeled with ^{99m}Tc. Its corresponding rhenium analogue was synthesized, and the fluorescent staining was performed in brain sections of both Tg mouse and Alzheimer's disease (AD) patient. The fluorescent rhenium complex Re-MAMA-BTA selectively bound to the amyloid aggregates in the brain sections of both APP Tg mouse and AD patient. The analogous ^{99m}Tc-MAMA-BTA complex could enter the normal mouse brain with high initial uptake. These results are encouraging for further exploration of their derivatives as imaging agents for Aβ plaques in the brain.

Alzheimer's disease (AD) is a progressive neurodegenerative disorder and a leading cause of dementia. AD is characterized by abundant senile plaques composed of β-amyloid (Aβ) peptide and numerous neurofibrillary tangles formed from filaments of highly phosphorylated tau proteins.[1,2] Formation of Aβ plaques in the brain is a pivotal event in the pathology of AD. Detection of deposited Aβ with non-invasive techniques such as positron emission tomography (PET) and single photon emission computed tomography (SPECT) could enable the diagnosis of AD in its pre-symptomatic stages.[3–6] Many specific ligands have been developed for imaging of Aβ aggregates. Out of the many agents that have been reported to target Aβ aggregates, some have so far moved forward into further evaluation studies using cohorts of AD patients: ^{18}F-FDDNP,[7,8] ^{11}C-PIB,[9,10] ^{11}C-SB-13,[11,12] and ^{11}C-BF-207.[13] The results from the initial studies are encouraging; and all of them are in clinical evaluation as PET imaging agents for AD.

However, the development of imaging agents for SPECT was lagging far behind, especially for the ^{99m}Tc-labeled radioactive probes, which were hindered by the low brain uptake. The most widely used radionuclide for diagnostic imaging with SPECT is the metastable isotope of technetium, ^{99m}Tc, because of its favorable physical properties ($t_{1/2}$ = 6 h, E_γ = 140 keV), low cost, and widespread availability.[14] The development of technetium complexes as potential radiopharmaceuticals is facilitated by the use of rhenium, the group VIIB congener of technetium. Rhenium generally produces complexes with similar physical and biodistribution properties to those of technetium and is often used as a non-radioactive alternative to technetium for large-scale synthesis and structural characterization.[15,16]

The ^{99m}Tc-labeled Congo red cannot penetrate the blood–brain barrier (BBB), probably because of its charge and high molecular weight.[17,18] The more lipophilic chrysamine-G has been labeled with ^{99m}Tc-monoamine-monoamide bisthiol (MAMA), but studies also demonstrated too low brain uptake.[19,20]

More lipophilic ligands with smaller molecular weight such as benzothiazole aniline (BTA),[21–24] stilbene, and biphenyl have been labeled with ^{99m}Tc, among which

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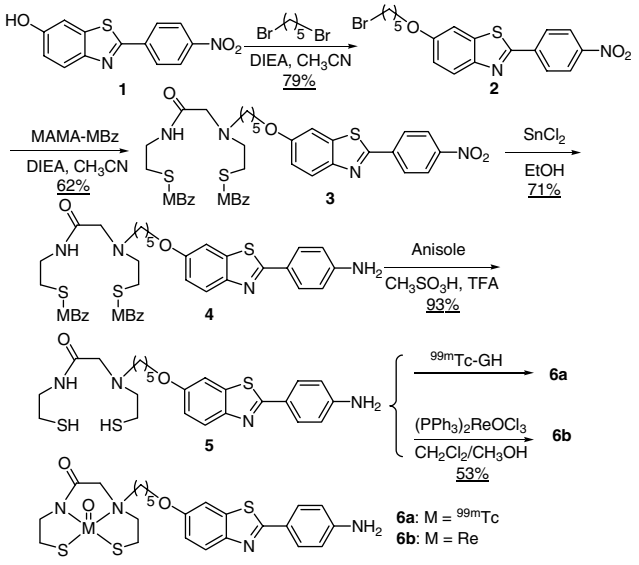
^{99m}Tc-labeled stilbene[25] and biphenyl[26] showed high brain uptake.

In our previous works, we investigated the possible binding mechanism and site of the ligands using molecular modeling techniques.[27] In this work, the chelator MAMA was conjugated with 2-(4-aminophenyl)benzothiazole through a 5-carbon alkyl chain as linker based on the structure of thioflavin-T and thioflavin-S, aiming at the development of analogous technetium and rhenium complexes with affinity for Aβ aggregates. ^{99m}Tc-MAMA complexes 6a (^{99m}Tc) and 6b (Re) were prepared, respectively, and biological characterization was also performed to evaluate their potential as tracer for visualization of β-amyloid plaques.

The compound 2-(4-nitrophenyl)-6-hydroxybenzothiazole (1) was conjugated with 1,5-dibromopentane to afford the compound 2 (yield 79%). Then the compound 2 was joined to the monoamine-monoamide bisthiol protected with *p*-methoxy benzyl (MAMA-MBz) to generate the compound 3 (yield 62%). After reduction of the nitro group using stannous chloride, the compound 4 (yield 71%) was obtained. Compound 5 (yield 93%) was obtained by deprotection of the thiol groups in compound 4. The complexes 6a and 6b (yield 53%) were prepared by the reaction of compound 5 with corresponding technetium and rhenium precursors, as outlined in Scheme 1.[28]

Biological evaluation of complex 6b was accomplished by fluorescent staining of brain sections of a Tg C57 (APP, 12-month-old) mouse and AD patient (male, 57 y) according to the reported method.[28,29] The complex 6b could bind the Aβ aggregates in Tg C57 (APP) mouse as shown in Figure 1A. Complex 6b also demonstrated binding affinity to different sizes of Aβ plaques in postmortem brain section of AD patient as shown in Figure 1B–D.

BTA is a kind of small molecule which has been proved to bind to amyloid with high affinity. According to our previous molecular modeling, the analogues of stilbene and thioflavin-T should be embedded in the amyloid when they bind to it.[27] In order to keep its binding affinity, the chelating moiety should be apart from benzothiazole moiety. The linker we chose is a 5-carbon alkyl chain, which is flexible and with the length of ~8 Å. So the interference on the interaction between BTA ligand and the amyloid by the chelating moiety is diminished. In addition, Re-MAMA-BTA bears similar structure as thioflavin-S, so it can be considered as a design of 'integrated method' based on thioflavin-S.



Scheme 1. [a]MBz = *p*-methoxy benzyl.

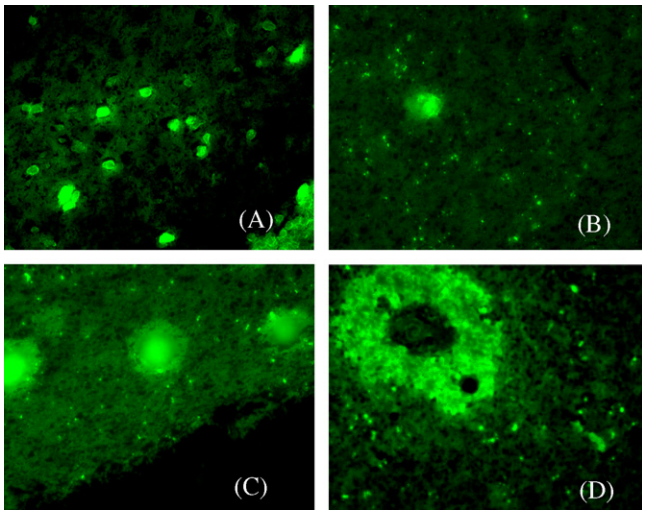


Figure 1. Fluorescence microscopy photographs showing the complex **6b** selectively stained Aβ aggregates in the Tg C57 (APP) mouse and AD patient brain sections: (A) Tg C57 (APP) mouse brain section with 3.5 mM of **6b** in 40% EtOH solution for 10 min. (B–D) Postmortem AD patient brain section with 3.5 mM of **6b** in 40% EtOH solution for 10 min.

The corresponding ^{99m}Tc complex of 6b, complex 6a, was also prepared by ligand exchange reaction employing the precursor ^{99m}Tc-glucoheptonate (GH). The labeling yield detected by radio-TLC was higher than 95%. The resulting mixture was analyzed by reversed-phase HPLC, showing that a single radioactive complex was formed with radio-chemical purity higher than 95% and the radioactive complex was stable for more than 12 h in PBS at room temperature. The identity of the radioactive complex was established by comparative HPLC studies using the corresponding rhenium complex 6b as reference. The retention times for 6b (UV) and 6a (radioactivity) on HPLC were 30.35 and 32.0 min, respectively. The complex 6a was rather hydrophobic with log*P* value 2.90 ± 0.13 measured using partition method between *n*-octanol and PBS.[28]

The biodistribution of complex 6a was carried out in IRC normal mice (18–22 g), which were sacrificed at the specific time after intravenous administration of the purified complex 1b (5–10 μCi).[28] The biodistribution data are shown in Table 1. The complex 6a showed high initial brain uptake (1.34 ± 0.16% ID/g organ at 2 min) and medium wash-out. The radioactivity was mainly excreted via the hepatobiliary system. The wash-out is to be expected since there is no amyloid in

Table 1. Biodistribution of radioactivity measured after the administration of complex **6a** in normal mice[a]

Organ	2 min	30 min	60 min	120 min
Blood	6.14 ± 0.47	3.88 ± 0.19	4.43 ± 0.56	4.71 ± 1.24
Heart	17.49 ± 0.70	5.31 ± 0.30	5.09 ± 0.75	3.75 ± 0.77
Lung	10.38 ± 0.87	5.03 ± 0.40	4.38 ± 0.39	3.34 ± 0.62
Liver	25.94 ± 2.24	34.51 ± 4.28	34.62 ± 5.98	32.45 ± 4.27
Spleen	4.60 ± 1.23	4.50 ± 0.86	3.51 ± 0.66	2.89 ± 0.49
Kidney	15.88 ± 1.58	8.92 ± 0.44	6.98 ± 1.33	5.10 ± 0.73
Brain	1.34 ± 0.16	0.99 ± 0.08	0.65 ± 0.10	0.44 ± 0.04

[a] All data are the mean percentage ($n = 3$) of the injected dose per gram of wet tissue (% ID/g) ± the standard deviation of the mean.

the normal mice brain. The high blood background, which may be due to the high hydrophobicity, is unfavorable for imaging application.

The lipophilicity plays an important role in BBB penetration. Hansch and Leo found that blood–brain barrier penetration is optimal when the log *P* values are in the range of 1.5–2.7, with the mean value of 2.1.[30] The ligands that can bind to Aβ aggregates are very hydrophobic and the hydrophobic interaction contributes to the binding. However, compounds with high log *P* value may bind to the proteins in the blood leading to high blood background. This contradiction seems not easy to be compromised. The less lipophilic MAMA ligand was chosen to adjust the lipophilicity of final compound. Former studies conjugating BTA with bisaminoethanethiol ligand showed lower brain uptake of the Tc-99m labeled compound.[21,24] And 6-Me-BTA lost its binding affinity after chelation with $^{99m}Tc(CO)_3$.[22] The choice of suitable conjugation method is critical for the binding affinity and brain uptake. Further experiments will be optimizing the pharmacokinetics while keeping its binding affinity. Introducing hydroxyl group to increase its solubility may improve the pharmacokinetics.

In conclusion, the isostructural ^{99m}Tc and Re complexes 6a and 6b were successfully synthesized and their preliminary evaluation results demonstrated the binding of 6b to Aβ aggregates in the brain slices of transgenic mouse and AD patient. In addition, complex 6a can penetrate BBB with high initial brain uptake and medium wash-out. These results are encouraging for further exploration of their derivatives as imaging agents for Aβ plaques in the brain.

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Supplementary data

Synthetic procedures and HPLC analysis for 6a and 6b, fluorescent staining of brain sections with 6b, partition coefficient determination and biodistribution of 6a in normal mice are available in supporting information. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.12.071.

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