2-(4'-Aminophenyl)benzothiazole Labeled with ^{99m}Tc -Cyclopentadienyl for Imaging β -Amyloid Plaques

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S Supporting Information

ABSTRACT: The development of a ^{99m}Tc -radiotracer for imaging of β -amyloid ($A\beta$) plaques with single photon emission computed tomography (SPECT) is strongly anticipated to provide a low cost and broadly accessible diagnostic tool for Alzheimer's disease (AD). Within this framework, 2-(4'-aminophenyl)benzothiazole, known to display affinity and specificity for $A\beta$ plaques, has been joined to the tricarbonyl *fac*- $[\text{M}(\text{CO})_3]^+$ ($\text{M} = \text{Re}(\text{I}), ^{99m}\text{Tc}(\text{I})$) core through the cyclopentadienyl moiety to yield stable, neutral, and lipophilic complexes (**Re-1** and $^{99m}\text{Tc-1}$, respectively). The **Re-1** complex was completely characterized with spectroscopic methods and was shown to selectively stain $A\beta$ plaques on sections of human AD brain tissue. The $^{99m}\text{Tc-1}$ complex displayed satisfactory initial brain uptake (0.53% ID/g at 2 min) and *in vivo* stability in healthy mice, while in transgenic 5xFAD mice, models for AD, a notable retention in the brain was noted (1.94% ID/g at 90 min). The results are encouraging and contribute to the effort of developing a SPECT amyloid imaging agent.

KEYWORDS: 2-(4'-Aminophenyl)benzothiazole, cyclopentadienyl, Re , ^{99m}Tc , β -amyloid plaques, SPECT imaging

Ever since the introduction of 2-(4'-[^{11}C]-methylaminophenyl)-6'-hydroxybenzothiazole (Pittsburgh compound-B, [^{11}C]PiB) for imaging of β -amyloid ($A\beta$) plaques of Alzheimer's disease (AD) with positron emission tomography (PET),¹ the development of a ^{99m}Tc -radiotracer for imaging of $A\beta$ plaques with single photon emission computed tomography (SPECT) has been widely sought after, in order to address the limitations of PET imaging in terms of cost and broad accessibility.² ^{99m}Tc has ideal nuclear properties ($t_{1/2}$ 6.01 h, E_{max} 141 keV) suitable for *in vivo* imaging and is readily available through portable $^{99}\text{Mo}/^{99m}\text{Tc}$ generators.³ It is therefore anticipated that a ^{99m}Tc -labeled imaging probe for $A\beta$ plaques will be of great clinical utility allowing the convenient and low cost radiodiagnosis of AD.

A variety of $A\beta$ plaque binding molecules (including derivatives of benzofuran, flavone, aurone, chalcone, benzothiazole, and benzoxazole), incorporation strategies through the conjugated or integrated approach, and ^{99m}Tc cores have been employed toward the generation of a compact and neutral ^{99m}Tc complex capable of crossing the blood–brain barrier (BBB) and selectively binding to $A\beta$ plaques.^{4–6} The ^{99m}Tc cores commonly utilized are the oxotechnetium [^{99m}TcO]³⁺ and the tricarbonyl *fac*- $[\text{M}(\text{CO})_3]^+$ along with its organometallic half-sandwich derivative the cyclopentadienyl tricarbonyl [$\text{Cp}^{99m}\text{Tc}(\text{CO})_3$] core.⁷ The [$\text{Cp}^{99m}\text{Tc}(\text{CO})_3$] labeling moiety has attractive properties for brain targeting radiopharmaceuticals, such as small size, low molecular weight, lipophilicity, stability, easy coupling to bioactive molecules,

and minimal steric interference with biological activity.⁸ It has been employed in the generation of potential AD imaging probes either through the integrated approach by mimicking the chalcone backbone⁹ or the conjugated approach by its attachment to a hydroxyl group on the phenylbenzothiazole moiety.^{6,10} Within the framework of our involvement with the applications of the 2-(4'-aminophenyl)benzothiazole pharmacophore in ^{99m}Tc -radioagents,^{11–13} we present herein a new complex (**1**, Scheme 1) in which the [$\text{CpM}(\text{CO})_3$] ($\text{M} = \text{Re}(\text{I}), ^{99m}\text{Tc}(\text{I})$) core has been joined to 2-(4'-aminophenyl)benzothiazole through the amine group, along with its initial biological assessment as β -amyloid imaging agent.

The **Re** analogue (**Re-1**) was synthesized first for characterization and biological evaluation experiments. The [$(\text{Cp-COOH})\text{Re}(\text{CO})_3$] complex (**2**, Scheme 1) was first activated with the HATU coupling reagent and then allowed to react with 2-(4'-aminophenyl)benzothiazole to give after purification the desired complex **Re-1**. The complex was characterized by IR and NMR spectroscopies as well as MS–ESI analysis. All relevant experimental data are given in Supporting Information. The affinity of **Re-1** for $A\beta$ plaques in brain sections from an AD patient was investigated with confocal microscopy, taking advantage of the strong fluorescence properties of the

Received: July 19, 2017

Accepted: September 17, 2017

Published: September 18, 2017

Scheme 1. Synthetic Route to Re (Re-1) and ^{99m}Tc ($^{99m}\text{Tc-1}$) Complexes^a

^aReagents/conditions: (a) 2-(4'-aminophenyl)benzothiazole, *N*-methylmorpholine, HATU, DMF, rt; (b) (i) oxalyl chloride, 0 °C (ii) 2-(4'-aminophenyl)benzothiazole, DIPEA, THF, rt; (c) Na[$^{99m}\text{TcO}_4$], Mn(CO)₅Br, DMF/H₂O, 110 °C, 60 min.

phenylbenzothiazole moiety.¹⁴ The images obtained (Figure 1A,B) clearly indicate that complex Re-1 specifically labels

Figure 1. Confocal fluorescence images (DAPI filter; excitation, 365 nm; emission, 445 nm) of staining with complex Re-1 (blue fluorescence) of brain slices from (A) an autopsy-confirmed AD patient and (C) from Tg 5xFAD mice. For comparison, corresponding brain slices (B,D) were stained with Thioflavin S (yellow-green fluorescence). The scale bar corresponds to 100 μm (A,B) and 50 μm (C,D).

amyloid plaques in a way similar to the clinically used dye Thioflavin S, as well as to other related phenylbenzothiazole complexes.^{6,10,13} The positive binding results prompted the transfer of chemistry at the ^{99m}Tc level.

The corresponding ^{99m}Tc complex, $^{99m}\text{Tc-1}$, was prepared by two methods from the ferrocene precursor 3, which was synthesized from coupling of the ferrocene carboxylic acid 4 with 2-(4'-aminophenyl)benzothiazole (Scheme 1). The first

method is a ligand exchange reaction employing the *fac*-[$^{99m}\text{Tc}(\text{H}_2\text{O})_3(\text{CO})_3$]⁺ complex (yield 35%) and the second one is a double ligand transfer (DLT) reaction employing Mn(CO)₅Br and Na[$^{99m}\text{TcO}_4$] (yield > 95%). The identity of the $^{99m}\text{Tc-1}$ complex was established by comparative HPLC using the corresponding Re complex as a reference (Figure S3, Supporting Information).

The $^{99m}\text{Tc-1}$ complex was stable (>95%) for a period of at least 6 h at room temperature and was also found stable (>90%) against the histidine and cysteine challenge for the same period of time (Table S2, Supporting Information). Its lipophilicity was determined by measuring its partition coefficient (*P*) in *n*-octanol/phosphate buffer (0.1 M, pH 7.4), which resulted in a $\log P_{\text{oct/water}}$ value of 2.35 ± 0.08 . This value is well within the range of $\log P_{\text{oct/water}} = 0.9\text{--}2.5$, which is considered suitable for a compound to be able to cross the blood–brain barrier and serve as brain pharmaceutical.¹⁵

The binding affinity of complex Re-1 for A β 42 aggregates was measured through an *in vitro* competition binding assay between the stable Re-1 and its radioactive $^{99m}\text{Tc-1}$ analogue, according to published procedures.¹⁶ As shown in Figure 2, the

Figure 2. Inhibition curve of Re-1 for the binding of $^{99m}\text{Tc-1}$ to A β 42 aggregates. Error bars represent standard deviation ($n = 3$).

presence of increasing concentrations of Re-1 inhibited the binding of $^{99m}\text{Tc-1}$ in a dose-dependent way that denotes the affinity of these complexes for A β 42 aggregates. The $K_i = 13.6 \pm 4.8$ nM calculated from the competitive inhibition data shows high binding affinity of Re-1 to the A β aggregates comparable to the one of [^{123}I]IMPY (12.5 ± 2.8 nM),¹⁰ the only SPECT A β imaging agent in preclinical trial, and is within the highest reported for potential A β SPECT imaging agents in the literature.^{6,10,17}

Biodistribution experiments of complex $^{99m}\text{Tc-1}$ in healthy Swiss albino mice (Table 1) revealed a moderate initial brain uptake of 0.53% ID/g at 2 min and relatively slow clearance of radioactivity from the brain with a ratio brain_{2 min}/brain_{90 min} of 2.1. The complex was efficiently cleared from blood, while the radioactivity was excreted by both the gastrointestinal and urinary tract. The low values of radioactivity in the spleen and the stomach are indicative of *in vivo* stability of the complex.¹⁸ Corresponding γ -camera imaging experiments in healthy mice (Figure S4, Supporting Information) with complex $^{99m}\text{Tc-1}$ gave comparable results showing accumulation in the liver, relatively slow clearance through the bladder, and 1.3% of total radioactivity in the brain at 15 min, which, taking into consideration the cumulative nature of planar imaging, is in accord with the biodistribution data.

Administration of $^{99m}\text{Tc-1}$ in 7-month-old transgenic (Tg) 5xFAD mice, which are models for Alzheimer's disease,¹⁹ was

Table 1. Biodistribution of Radioactivity after Injection of Complex ^{99m}Tc -1 in Healthy Swiss Albino Mice ($n = 4$) and in Transgenic Mice Models for AD ($n = 3$)^a

organ	% ID/g healthy mice			% ID/g Tg 5xFAD	
	2 min	15 min	90 min	2 min	90 min
blood	15.00 $\pm$ 2.78	2.86 $\pm$ 0.19	1.77 $\pm$ 0.03	23.47 $\pm$ 1.36	5.84 $\pm$ 1.06
liver	15.76 $\pm$ 3.16	36.34 $\pm$ 5.76	31.77 $\pm$ 2.49	24.12 $\pm$ 1.30	34.72 $\pm$ 5.41
heart	5.79 $\pm$ 1.83	2.55 $\pm$ 0.56	1.46 $\pm$ 0.07	7.34 $\pm$ 0.73	8.84 $\pm$ 0.28
kidneys	7.42 $\pm$ 1.46	6.61 $\pm$ 0.98	10.31 $\pm$ 1.52	11.21 $\pm$ 1.36	19.91 $\pm$ 3.06
stomach	0.55 $\pm$ 0.19	0.91 $\pm$ 0.07	2.34 $\pm$ 1.30	1.02 $\pm$ 0.57	0.95 $\pm$ 0.17
intestines	0.66 $\pm$ 0.06	1.29 $\pm$ 0.42	6.64 $\pm$ 1.80	0.87 $\pm$ 0.06	6.06 $\pm$ 0.71
spleen	23.51 $\pm$ 7.40	31.73 $\pm$ 3.26	3.22 $\pm$ 0.68	12.74 $\pm$ 2.31	23.13 $\pm$ 0.34
muscle	0.76 $\pm$ 0.20	1.18 $\pm$ 0.67	0.37 $\pm$ 0.03	0.62 $\pm$ 0.07	0.14 $\pm$ 0.01
lungs	42.04 $\pm$ 5.14	17.65 $\pm$ 1.48	4.06 $\pm$ 0.45	26.45 $\pm$ 2.40	8.51 $\pm$ 0.39
pancreas	1.70 $\pm$ 0.41	1.60 $\pm$ 0.18	1.10 $\pm$ 0.20	1.96 $\pm$ 0.89	4.17 $\pm$ 0.65
brain	0.53 $\pm$ 0.11	0.34 $\pm$ 0.01	0.25 $\pm$ 0.08	0.52 $\pm$ 0.08	1.94 $\pm$ 0.26

^aAll data are expressed as the % of the injected dose per gram of wet tissue (% ID/g) $\pm$ the standard deviation of the mean.

subsequently affected in order to determine whether increased brain uptake/retention is noted in the pathological brain that would be in the right direction for imaging applications. The results of biodistribution are shown in Table 1 where 0.52% ID/g of radioactivity is recorded in the brain at 2 min, a result similar to that in healthy mice. However, at 90 min a notable 1.94% ID/g is found, a result that, in combination with the drop of radioactivity in the blood, suggests the complex is retained in the brain. Figure 3 shows static images of the Tg 5xFAD mouse obtained at 15, 30, and 60 min, while in Figure S5 the % radioactivity present in the various organs is plotted vs time. In the imaging experiment, the brain radioactivity was measured at 60 min to be 3.33%, which again is judged as

significant considering the fact that the radioactivity in the blood at 60 min has significantly dropped.

Biodistribution experiments were also performed in non-transgenic (wild type) littermates of the Tg 5xFAD mice to serve as control, and the results are presented in Supporting Information Table S3. The uptake (0.42% ID/g) and clearance rate values (brain_{2 min}/brain_{90 min} of 2.0) are essentially the same as those recorded for the healthy mice (Table 1) and suggest that the entrapment of radioactivity in the brain of the transgenic mice can reasonably be ascribed to the presence of amyloid plaques. To further confirm the presence of plaques, the brain was removed, treated with formol, cut into slices, and subjected to staining with both Re-1 and Thioflavin S. The results are shown in Figure 1C,D where it is confirmed that the brain of transgenic mice carry a heavy load of amyloid plaques.

In conclusion, a new cyclopentadienyl complex 1 ($M = \text{Re}$, ^{99m}Tc) joined to 2-(4'-aminophenyl)benzothiazole through formation of an amide bond is presented as potential β -amyloid imaging agent. In complex Re-1, the affinity of the 2-(4'-aminophenyl)benzothiazole for amyloid plaques is maintained as evidenced by staining plaques from an AD patient as well as from Tg 5xFAD mice models for AD. Complex ^{99m}Tc -1 has a relatively small MW of 500 Da and displays excellent stability and ideal lipophilicity as well as high *in vitro* affinity ($K_i = 13.6$ nM) for amyloid fibrils. Its moderate brain uptake (0.53% ID/g at 2 min) is within the range of those reported (0.26–1.06% ID/g) for other cyclopentadienyl complexes of 2-(4'-aminophenyl)benzothiazole.^{6,10} The significant increase, however, of radioactivity in the brain of Tg 5xFAD mice with time (1.94% ID/g at 90 min post injection) is consistent with retention of ^{99m}Tc -1 through binding to β -amyloid plaques. Even though ^{99m}Tc complexes of higher initial uptake have been reported in the literature,⁵ the intricate balance of requirements that a new agent should display in order to be considered for application⁸ makes this compact and lipophilic complex a promising structural alternative worth of further fine-tuning to meet the prerequisites of a SPECT amyloid imaging agent. Moreover, the diverse pharmacological spectrum of 2-substituted benzothiazoles (including cancer, inflammation, microbes, neurological disorders^{20–22}) justifies the further exploration of potential applications of ^{99m}Tc -1 and its rhenium analogue Re-1.

Figure 3. Static γ -images (2 min duration) of a Tg 5xFAD mouse at 15, 30, and 60 min after injection of complex ^{99m}Tc -1.

■ ASSOCIATED CONTENT

■ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmmedchem-lett.7b00294.

Experimental details for synthesis and characterization of ferrocene precursor **3** and complexes **Re-1** and ^{99m}Tc-**1**, including ¹H and ¹³C NMR spectroscopic data; comparative HPLC for **Re-1** and ^{99m}Tc-**1**; stability studies of ^{99m}Tc-**1**; binding studies using Aβ42 fibrillar aggregates and *in vitro* binding of **Re-1** to amyloid plaques; biodistribution and imaging studies (PDF)

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors thankfully acknowledge the generous contribution of Professor David Mann of the Greater Manchester Neurosciences Centre, University of Manchester in the AD brain tissue staining experiments. They also thank Dr. Athina Tzinia of the Laboratory of Cell & Matrix Biochemistry/Pathobiology, Institute of Biosciences & Applications, NCSR "Demokritos", for kindly providing the 5xFAD transgenic mice.

■ REFERENCES

- (1) Klunk, W. E.; Engler, H.; Nordberg, A.; Wang, Y.; Blomqvist, G.; Holt, D. P.; Bergstrom, M.; Savitcheva, I.; Huang, G. F.; Estrada, S.; Ausen, B.; Debnath, M. L.; Barletta, J.; Price, J. C.; Sandell, J.; Lopresti, B. J.; Wall, A.; Koivisto, P.; Antoni, G.; Mathis, C. A.; Langstrom, B. Imaging brain amyloid in Alzheimer's disease with Pittsburgh compound-B. *Ann. Neurol.* **2004**, *55*, 306–319.
- (2) Bateman, T. M. Advantages and disadvantages of PET and SPECT in a busy clinical practice. *J. Nucl. Cardiol.* **2012**, *19*, S3–S11.
- (3) Bartholoma, M. D.; Louie, A. S.; Valliant, J. F.; Zubieta, J. Technetium and gallium derived radiopharmaceuticals: comparing and contrasting the chemistry of two important radiometals for the molecular imaging era. *Chem. Rev.* **2010**, *110*, 2903–2920.
- (4) Ono, M.; Saji, H. Recent advances in molecular imaging probes for beta-amyloid plaques. *MedChemComm* **2015**, *6*, 391–402.
- (5) Zhang, X.; Yu, P.; Yang, Y.; Hou, Y.; Peng, C.; Liang, Z.; Lu, J.; Chen, B.; Dai, J.; Liu, B.; Cui, M. ^{99m}Tc-Labeled 2-Arylbenzothiazoles: Abeta Imaging Probes with Favorable Brain Pharmacokinetics for Single-Photon Emission Computed Tomography. *Bioconjugate Chem.* **2016**, *27*, 2493–2504.
- (6) Jia, J.; Zhou, K.; Dai, J.; Liu, B.; Cui, M. 2-Arylbenzothiazoles labeled with [CpRe/^{99m}Tc(CO)₃] and evaluated as beta-amyloid imaging probes. *Eur. J. Med. Chem.* **2016**, *124*, 763–772.
- (7) Bernard, J.; Ortner, K.; Spingler, B.; Pietzsch, H. J.; Alberto, R. Aqueous synthesis of derivatized cyclopentadienyl complexes of technetium and rhenium directed toward radiopharmaceutical application. *Inorg. Chem.* **2003**, *42*, 1014–1022.
- (8) Cui, M. Past and recent progress of molecular imaging probes for beta-amyloid plaques in the brain. *Curr. Med. Chem.* **2014**, *21*, 82–112.
- (9) Li, Z.; Cui, M.; Dai, J.; Wang, X.; Yu, P.; Yang, Y.; Jia, J.; Fu, H.; Ono, M.; Jia, H.; Saji, H.; Liu, B. Novel cyclopentadienyl tricarbonyl complexes of (^{99m}Tc) mimicking chalcone as potential single-photon emission computed tomography imaging probes for beta-amyloid plaques in brain. *J. Med. Chem.* **2013**, *56*, 471–482.
- (10) Jia, J.; Cui, M.; Dai, J.; Liu, B. 2-Phenylbenzothiazole conjugated with cyclopentadienyl tricarbonyl [CpM(CO)₃] (M = Re, (^{99m}Tc)) complexes as potential imaging probes for beta-amyloid plaques. *Dalton Trans.* **2015**, *44*, 6406–6415.
- (11) Tzanopoulou, S.; Sagnou, M.; Paravatou-Petsotas, M.; Gourni, E.; Loudos, G.; Xanthopoulos, S.; Lafkas, D.; Kiaris, H.; Varvarigou, A.; Pirmettis, I. C.; Papadopoulos, M.; Pelecanou, M. Evaluation of Re and (^{99m}Tc) complexes of 2-(4'-aminophenyl)benzothiazole as potential breast cancer radiopharmaceuticals. *J. Med. Chem.* **2010**, *53*, 4633–4641.
- (12) Tzanopoulou, S.; Pirmettis, I. C.; Patsis, G.; Paravatou-Petsotas, M.; Livaniou, E.; Papadopoulos, M.; Pelecanou, M. Synthesis, characterization, and biological evaluation of M(I)(CO)(3)(NNO) complexes (M = Re, Tc-^{99m}) conjugated to 2-(4-aminophenyl)-benzothiazole as potential breast cancer radiopharmaceuticals. *J. Med. Chem.* **2006**, *49*, 5408–5410.
- (13) Sagnou, M.; Tzanopoulou, S.; Raptopoulou, C. P.; Psycharis, V.; Braband, H.; Alberto, R.; Pirmettis, I. C.; Papadopoulos, M.; Pelecanou, M. A Phenylbenzothiazole Conjugate with the Tricarbonyl fac-[M(I)(CO)(3)](+) (M = Re, Tc-^{99m}, Tc-^{99m}) Core for Imaging of beta-Amyloid Plaques. *Eur. J. Inorg. Chem.* **2012**, *2012*, 4279–4286.
- (14) Dey, J.; Dogra, S. K. Dual Fluorescence of 2-(4'-(N,N-Dimethylamino)Phenyl)Benzothiazole and Its Benzimidazole Analog - Effect of Solvent and pH on Electronic-Spectra. *J. Phys. Chem.* **1994**, *98*, 3638–3644.
- (15) Dischino, D. D.; Welch, M. J.; Kilbourn, M. R.; Raichle, M. E. Relationship between Lipophilicity and Brain Extraction of C-11-Labeled Radiopharmaceuticals. *J. Nucl. Med.* **1983**, *24*, 1030–1038.
- (16) Klunk, W. E.; Wang, Y.; Huang, G. F.; Debnath, M. L.; Holt, D. P.; Mathis, C. A. Uncharged thioflavin-T derivatives bind to amyloid-beta protein with high affinity and readily enter the brain. *Life Sci.* **2001**, *69*, 1471–1484.
- (17) Yang, Y.; Cui, M.; Jin, B.; Wang, X.; Li, Z.; Yu, P.; Jia, J.; Fu, H.; Jia, H.; Liu, B. (^{99m}Tc)-labeled dibenzylideneacetone derivatives as potential SPECT probes for *in vivo* imaging of beta-amyloid plaque. *Eur. J. Med. Chem.* **2013**, *64*, 90–98.
- (18) Yazdani, A.; Janzen, N.; Czorny, S.; Valliant, J. F. Technetium(I) Complexes of Bathophenanthrolinedisulfonic Acid. *Inorg. Chem.* **2017**, *56*, 2958–2965.
- (19) Oakley, H.; Cole, S. L.; Logan, S.; Maus, E.; Shao, P.; Craft, J.; Guillozet-Bongaarts, A.; Ohno, M.; Disterhoft, J.; Van Eldik, L.; Berry, R.; Vassar, R. Intraneuronal beta-amyloid aggregates, neurodegeneration, and neuron loss in transgenic mice with five familial Alzheimer's disease mutations: potential factors in amyloid plaque formation. *J. Neurosci.* **2006**, *26*, 10129–10140.
- (20) Jiang, L.; Zhang, M. K.; Tang, L.; Weng, Q. J.; Shen, Y. H.; Hu, Y. Z.; Sheng, R. Identification of 2-substituted benzothiazole derivatives as triple-functional agents with potential for AD therapy. *RSC Adv.* **2016**, *6*, 17318–17327.
- (21) Hroch, L.; Aitken, L.; Benek, O.; Dolezal, M.; Kuca, K.; Gunn-Moore, F.; Musilek, K. Benzothiazoles - Scaffold of Interest for CNS Targeted Drugs. *Curr. Med. Chem.* **2015**, *22*, 730–747.
- (22) Gagonia, J.; Verma, P. K. GABA-A Inhibitory Activity of Benzothiazole - GABA Analogs: In-Silico Approach for Antiepileptic Profile. *Int. J. Pharm. Sci. Res.* **2016**, *7*, 4437–4442.