SPECT Imaging Agents for Detecting Cerebral β-Amyloid Plaques

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1. Introduction

Alzheimer’s disease (AD) is an age-related, irreversible form of dementia characterized by memory loss, a progressive decline in intellectual ability, language impairment, and personality and behavioral changes that eventually interfere with daily life. The accumulation of β-amyloid (Aβ) aggregates (major protein aggregates of senile plaques) in the brain is considered one of the hallmarks of AD [1, 2]. Today, the clinical diagnosis of AD is primarily based on history and memory testing, which is often difficult and not accurate, as the early cognitive and behavioral symptoms of AD are difficult to distinguish from normal signs of aging. To facilitate the early diagnosis of this disease, there is an urgent need for the sensitive noninvasive detection of biomarkers for the pathophysiology. Toward achieving this goal, nuclear imaging techniques such as positron emission computed tomography (PET) and single photon emission computed tomography (SPECT) have been employed. Radionuclide-labeled agents targeting the Aβ plaques in the brain may greatly facilitate the diagnosis of AD and new antiamyloid therapies [3–7]. The differential diagnosis for AD includes a large number of other diseases such as vascular dementia, frontal temporal lobe dementia (FTLD) complex, and dementia with Lewy bodies (DLB) as well as rarer neurodegenerative diseases such as Creutzfeld-Jacob disease (CJD). Importantly, AD subjects will always have Aβ plaques, whereas Aβ is seen not at all or only sporadically in most of these other diseases. In each case, appropriate prognosis and treatment require accurate diagnostic assessment.

Developing Aβ imaging agents is currently an emerging field of research. The basic requirements for suitable Aβ imaging agents include (i) good penetration of the blood-brain barrier, (ii) selective binding to Aβ plaques, and (iii) clear and contrasting signals between plaques and nonplaques (Figure 1). Based on these requirements, several promising agents with the backbone structure of DDNP, thioflavin-T and Congo Red have been synthesized and evaluated for use in vivo as probes to image Aβ plaques in AD brain. Clinical trials in AD patients have been conducted with several PET imaging agents including [11C]PIB [8–10], [11C]SB-13 [6, 11], [11C]BF-227 [12], [11C]AZD2184 [13], [18F]FDDNP [14–16], [18F]BAY94-9172 [7, 17, 18], [18F]AV-45 [19–21], and [18F]GE-067 [22] (Figure 2), indicating the imaging of Aβ plaques in living brain tissue to be useful for the diagnosis of AD. The 11C-labeled agents limit their use to on-site cyclotrons and sophisticated radiochemistry laboratories due to the short half-life (20 min) of 11C. PET agents with the longer half-life (110 min) radioisotope 18F have recently been developed and could increase the availability of Aβ imaging to all PET facilities, but still represents a minority of modern hospitals, as only a small fraction of hospitals have a PET scanner. 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. However, progress in developing imaging agents targeting Aβ plaques is less advanced for SPECT than PET. In this review, we summarize the current situation in the development of probes for SPECT-based imaging of Aβ plaques in Alzheimer’s brains.

2. Radioiodinated Probes for Imaging of Aβ Plaques

Many radioiodinated imaging agents derived from Congo Red or thioflavin-T have been developed. Compounds 1 [29], 2 [29], 3 [40], 4 [23], 5 [24], and 6 [25] (Figure 3) are thought to be derived from Congo Red. Although 1, 2, and 3 showed unfavorable pharmacokinetics in vivo such as low uptake into the brain and a slow washout, the radioactivity pharmacokinetics of 5 and 6 was much improved. Because thioflavin-T has a lower molecular weight than Congo Red, implying greater blood-brain penetration, a number of groups have worked to develop probes for SPECT derived from thioflavin-T including 7 (IMPY) [26–28], 8 (TZDM) [29], 9 (IBOX) [30], 10 (benzofuran derivatives) [31], and 11 (phenylindole derivatives) (Figure 4) [32].

Initially, Zhuang and coworkers prepared iodostrylbenzene derivatives based on the chemical structure of Congo Red, [125I]IMSB (1) and [125I]ISB (2). These ligands exhibited low brain uptake likely due to two ionizable carboxyl groups [29]. Thus, a small and neutral thioflavin-T analog, [125I]TZDM (8), was prepared [29]. In vitro binding studies of these ligands, [125I]ISB, [125I]IMSB and [125I]TZDM, showed excellent binding affinities with $K_d$ values of 0.08, 0.13 and 0.06 nM for aggregates of Aβ(1–40) and 0.15, 0.73 and 0.14 nM for aggregates of Aβ(1–42), respectively. Interestingly, in a competitive-binding assay, different binding sites on Aβ(1–40) and Aβ(1–42) aggregates, which are mutually exclusive, were observed for Congo Red and thioflavin-T derivatives. Biodistribution experiments in normal mice after an i.v. injection showed that [125I]TZDM exhibited good uptake and retention in the brain, much higher than [125I]ISB and [125I]IMSB. Preliminary experiments on the biodistribution of [125I]TZDM in transgenic mice, engineered to produce excess Aβ plaques in the brain as an AD model, suggested labeling of Aβ aggregates in vivo. However, [125I]TZDM is not ideal as an imaging agent in vivo, due to its labeling of white matter, which significantly increases the background.
activity. To improve the pharmacokinetics of uptake and retention, Kung et al. and others have prepared several compounds derived from thioflavin-T and studied features such as affinity for Aβ aggregates in vitro and biodistribution in vivo. Interestingly, the compounds with good binding to Aβ aggregates share a common structural feature: either an N-methylamino- or N,N-dimethylaminophenyl group at one end of the molecule. The structural feature required for binding to Aβ aggregates appears to be simple and unique.

[123I]IMPY has been characterized as a potential agent for SPECT-based imaging of Aβ plaques. IMPY displayed selective labeling of Aβ plaques ex vivo in autoradiographic experiments using double-transgenic mice (PSAPP) as a model of AD [33]. Preliminary clinical data on [125I]IMPY in normal and AD patients showed a distinct distribution pattern similar to that of [11C]PIB [34, 35]. However, the signal-to-noise ratio for plaque labeling is not as high as that of [11C]PIB. The low contrast may be due to the fast clearance from brain and plasma observed in AD and normal subjects. But the rapid metabolism and instability of [123I]IMPY in vivo may have led to less than optimal signal-to-noise ratios for targeting Aβ plaques in the brain. Additional candidates are being explored for SPECT imaging of Aβ plaques in the brain.

Recently, the effects of polyhydroxyflavones on the formation, extension, and destabilization of Aβ aggregates have been studied in vitro [36]. These flavones dose-dependently inhibited the formation of Aβ aggregates, as well as destabilized preformed Aβ aggregates, indicating that they could interact directly with the aggregates. The findings in that report prompted us to use flavones as a core structure in the development of Aβ imaging agents. Furthermore, some recent studies have shown that electron-donating groups such as methylamino, dimethylamino, methoxy, and hydroxy groups play a critical role in the binding to Aβ aggregates. With these considerations in mind, we designed
four radioiodinated flavones with a radioiodine at the 6-position and an electron-donating group at the 4′-position (Figure 5). Then we synthesized a series of flavone derivatives and evaluated their usefulness in vivo as SPECT Aβ imaging agents [37].

Experiments on the binding of [125I]12 to aggregates of Aβ(1–40) and Aβ(1–42) were carried out. Transformation of the saturation binding of [125I]12 to Scatchard plots gave linear plots, suggesting one binding site (Figure 6). [125I]12 showed excellent affinity for both Aβ(1–40) (Kd = 12.4 ± 2.3 nM) and Aβ(1–42) (Kd = 17.4 ± 5.7 nM) aggregates. The binding of nonradioactive flavone derivatives (compounds 12, 13, 14, and 15) was evaluated in experiments inhibiting [125I]12 from binding Aβ(1–40) and Aβ(1–42) aggregates. As shown in Table 1, all flavone derivatives competed well with [125I]12 (Ki = 13–77 nM). More interestingly, when thioflavin-T and Congo Red gave high Ki values (>1000 nM) (Table 1), indicating little competition. This finding suggests that these flavones may have a binding site on Aβ aggregates different from that of thioflavin T and Congo Red, although additional studies regarding the selectivity of binding affinity for Aβ aggregates are required.

Since the in vitro binding assays demonstrated the high affinity of the flavone derivatives for Aβ(1–40) and Aβ(1–42) aggregates, compounds 12, 13, 14, and 15 were investigated for their neuropathologic staining of Aβ plaques and NFTs in human AD brain sections (Figure 7). The compounds intensely stained Aβ plaques (Figures 7(a), 7(e), 7(i), and 7(m)), neuritic plaques (Figures 7(b), 7(f), 7(j), and 7(n)), and cerebrovascular amyloids (Figures 7(c), 7(g), 7(k), and 7(o)) with nearly the same pattern. However, as seen in Figures 7(a), 7(e), 7(i), and 7(m), these flavone compounds did not intensely stain the core region in so-called classic Aβ plaques, unlike the thioflavin-T and Congo Red derivatives previously reported as Aβ imaging probes, indicating that flavone derivatives may have somewhat distinct binding characteristics for amyloid fibrils. These flavone derivatives appear to stain not only neuritic Aβ plaques but also diffuse amyloid plaque deposits, which are known to be mainly composed of Aβ(1–42) [38] and to be the initial pathologic change in AD [39]. Thus flavone derivatives with high affinity for Aβ(1–42)-positive diffuse plaques may be more useful for presymptomatic detection of AD. Furthermore, 12, 13, 14, and 15 also showed high affinity for NFTs in AD brain sections (Figures 7(d), 7(h), 7(l), and 7(p)). These findings suggest that these flavone derivatives can bind amyloid fibrils and NFTs without the backbone structure of thioflavin-T or Congo Red and that quantitative evaluation of their cerebral

### Table 1: Inhibition constants (Ki, nM) of compounds for the binding of ligands to aggregates of Aβ(1–40) and Aβ(1–42).

| Compound     | Aβ(1–40) | Aβ(1–42) |
|--------------|----------|----------|
| 12           | 22.6 ± 3.4 | 30.0 ± 3.4 |
| 13           | 13.2 ± 0.2 | 15.6 ± 2.4 |
| 14           | 29.0 ± 3.2 | 38.3 ± 8.1 |
| 15           | 72.5 ± 8.2 | 77.2 ± 9.2 |
| Thioflavin T | >1000    | >1000    |
| Congo Red    | >1000    | >1000    |

Values are the mean ± standard error of the mean for 6 independent experiments.
Figure 7: Neuropathological staining of compounds 12 (a)–(d), 13 (e)–(h), 14 (i)–(l), and 15 (m)–(p) on 5 μm AD brain sections from the temporal cortex. (a) Aβ plaques (a), (e), (i), and (m) are clearly stained with 12, 13, 14, and 15 (×40 magnification). Clear staining of neuritic plaques (b), (f), (j), and (n) and cerebrovascular amyloid (c), (g), (k), and (o) was also obtained. Many NFTs (d), (h), (l), and (p) are intensely stained with 12, 13, 14, and 15 (×40 magnification).

Figure 8: Chemical structure of diphenyl oxadiazoles.

Localization may provide useful information on Aβ and tau pathology.

Four radioiodinated flavone ligands ([125I]12, [125I]13, [125I]14, and [125I]15) were evaluated for their biodistribution in vivo in normal mice. Previous studies suggest that the optimal lipophilicity range for brain entry is observed for compounds with log P-values between 1 and 3 [5]. All four ligands displayed optimal lipophilicity as reflected by log P-values of 1.94, 2.69, 2.41, and 1.92, respectively. As expected, these ligands exhibited high uptake ranging from 3.2% to 4.1% ID/g brain at 2 min postinjection, a level sufficient for imaging in the brain (Table 2). In addition, they displayed good clearance from the normal brain: 1.2, 1.0, 0.17, and 0.08% ID/g at 60 min postinjection for [125I]12, [125I]13, [125I]14, and [125I]15, respectively. Radioiodinated amyloid imaging agents such as [125I]m-I-stilbene (3) [40], [125I]TZDM (8) [29], [125I]IBOX (9) [30], and [125I]benzofuran (10) [31], and [125I]phenylindole (11) [32] reported previously showed good uptake, but a relatively slow washout from the normal brain. A low washout rate leads to high background activity and prevents the visualization of Aβ plaques in the AD brain. Appropriate properties in vivo (higher uptake and faster washout from the normal brain) make radioiodinated flavones useful candidates for SPECT tracers for Aβ imaging.

On the basis of this success in the development of SPECT imaging agents, to search for more useful candidates for Aβ imaging probes, we have designed a chemical modification of the flavone structure, and selected the chalcone and aurone structure as a novel core for Aβ imaging probes (Figure 5) [41, 42]. Chalcone and aurone are categorized as flavonoids containing a flavone. We newly designed and synthesized novel chalcone and aurone derivatives, and evaluated the
effect of their structure–activity relationships on binding to Aβ aggregates and biodistribution in vivo using a compound with high affinity [42–45]. Currently, SPECT imaging agents based on chalcone and aurone are optimized.

Most of the Aβ imaging probes reported previously have two aromatic rings. Among them, 1,4-diphenyltriazole and 2,5-diphenylthiophene derivatives have triazole and thiophene between two benzene rings, respectively, and it has been shown that they have high-binding affinity for binding to Aβ aggregates despite the kinds of substituted groups [46, 47]. In an attempt to further develop novel ligands for the imaging of Aβ plaques in AD, we designed a series of 3,5-diphenyl-1,2,4-oxadiazole (18) [48, 49] and 2,4-diphenyl-1,3,5-oxadiazole (19) [49] derivatives (Figure 8). Although

Figure 9: Chemical structure of $^{99m}$Tc complexes for imaging of Aβ plaques.

Figure 10: Chemical structure of $^{99m}$Tc complexes based on chalcone (24), flavone (25), and aurone (26) for imaging of Aβ plaques.
Table 2: Biodistribution of radioactivity after intravenous injection of \[^{125}\text{I}]12, \[^{125}\text{I}]13, \[^{125}\text{I}]14, \text{ and }[^{125}\text{I}]15\) in normal mice\(^{a}\)

| Tissue       | 2               | 10              | 30              | 60              |
|--------------|-----------------|-----------------|-----------------|-----------------|
|              | \[^{125}\text{I}]12\) |                |                 |                 |
| Blood        | 1.89 (0.28)     | 1.39 (0.10)     | 1.34 (0.07)     | 1.50 (0.09)     |
| Liver        | 16.28 (0.90)    | 25.28 (0.31)    | 18.61 (1.81)    | 15.14 (0.89)    |
| Kidney       | 8.13 (1.28)     | 5.21 (0.44)     | 3.85 (0.33)     | 3.05 (0.25)     |
| Intestine    | 3.10 (0.61)     | 7.91 (1.05)     | 12.84 (1.18)    | 21.48 (3.17)    |
| Spleen       | 2.57 (1.54)     | 2.31 (0.01)     | 1.76 (0.23)     | 1.52 (0.29)     |
| Heart        | 4.87 (0.66)     | 2.66 (0.12)     | 1.67 (0.14)     | 1.28 (0.12)     |
| Stomach\(^{b}\)| 0.78 (0.02)   | 0.87 (0.22)     | 1.44 (0.69)     | 1.80 (0.84)     |
| Brain        | 4.12 (0.15)     | 3.68 (0.18)     | 1.84 (0.12)     | 1.19 (0.04)     |
|              | \[^{125}\text{I}]13\) |                |                 |                 |
| Blood        | 1.87 (0.18)     | 1.07 (0.08)     | 1.20 (0.15)     | 1.15 (0.16)     |
| Liver        | 15.41 (0.98)    | 21.85 (2.14)    | 15.71 (0.96)    | 12.40 (2.38)    |
| Kidney       | 8.33 (1.47)     | 4.31 (0.28)     | 3.40 (0.31)     | 2.32 (0.45)     |
| Intestine    | 2.24 (0.24)     | 6.56 (0.83)     | 12.97 (1.15)    | 18.64 (2.05)    |
| Spleen       | 2.72 (0.20)     | 1.92 (0.33)     | 1.58 (0.31)     | 1.18 (0.17)     |
| Heart        | 5.63 (0.80)     | 2.47 (0.14)     | 1.69 (0.06)     | 1.07 (0.17)     |
| Stomach\(^{b}\)| 0.73 (0.17) | 0.63 (0.16)     | 1.17 (0.40)     | 1.06 (0.27)     |
| Brain        | 3.22 (0.15)     | 3.61 (0.60)     | 1.89 (0.21)     | 0.99 (0.10)     |
|              | \[^{125}\text{I}]14\) |                |                 |                 |
| Blood        | 1.87 (0.21)     | 1.19 (0.17)     | 0.40 (0.01)     | 0.23 (0.09)     |
| Liver        | 8.96 (1.48)     | 9.01 (0.97)     | 3.75 (0.47)     | 1.88 (0.61)     |
| Kidney       | 7.99 (1.08)     | 6.30 (1.02)     | 4.51 (1.59)     | 1.46 (1.12)     |
| Intestine    | 3.52 (0.29)     | 14.39 (0.80)    | 22.51 (1.11)    | 30.05 (3.61)    |
| Spleen       | 2.70 (0.08)     | 1.38 (0.37)     | 0.55 (0.30)     | 3.67 (5.89)     |
| Heart        | 4.98 (0.41)     | 2.25 (0.40)     | 0.84 (0.14)     | 0.47 (0.22)     |
| Stomach\(^{b}\)| 0.68 (0.06) | 0.45 (0.18)     | 0.55 (0.33)     | 0.31 (0.07)     |
| Brain        | 4.00 (0.18)     | 2.36 (0.33)     | 0.51 (0.07)     | 0.17 (0.05)     |
|              | \[^{125}\text{I}]15\) |                |                 |                 |
| Blood        | 2.77 (0.43)     | 1.58 (0.18)     | 0.66 (0.03)     | 0.20 (0.02)     |
| Liver        | 9.77 (1.89)     | 8.24 (0.50)     | 6.80 (0.86)     | 4.78 (1.09)     |
| Kidney       | 14.79 (2.59)    | 15.11 (2.00)    | 6.45 (0.84)     | 1.66 (0.62)     |
| Intestine    | 3.12 (0.37)     | 11.26 (0.63)    | 22.01 (1.34)    | 27.28 (0.48)    |
| Spleen       | 3.92 (1.18)     | 1.55 (0.15)     | 0.56 (0.13)     | 0.17 (0.06)     |
| Heart        | 5.51 (0.71)     | 1.60 (0.18)     | 0.53 (0.04)     | 0.12 (0.02)     |
| Stomach\(^{b}\)| 0.89 (0.09) | 0.59 (0.16)     | 1.56 (0.50)     | 0.81 (0.36)     |
| Brain        | 3.31 (0.32)     | 1.90 (0.07)     | 0.52 (0.03)     | 0.08 (0.02)     |

\(^{a}\)Expressed as % injected dose per gram. Each value represents the mean ± S.D. for 3–5 animals at each interval. \(^{b}\)Expressed as % injected dose per organ.

the diphenyloxadiazole pharmacophore with high-binding affinity for A\(\beta\) aggregates may be useful as a backbone structure to develop novel A\(\beta\) imaging agents, additional modifications are necessary to improve the uptake and rapid clearance of nonspecifically bound radiotracers.

Many factors such as molecular size, ionic charge, and lipophilicity affect the brain uptake of compounds. Since lipophilicity of the compounds generally increases by introduction of iodine, the large higher lipophilicity of the radioiodinated compounds may constitute one reason for the low brain uptake. In the future, introduction of hydrophilic substituted groups into the amyloid-binding scaffolds will be required to develop more promising radioiodinated tracers with in favorable in vivo pharmacokinetics.

3. \(^{99m}\text{Tc}\) Complexes for Imaging of A\(\beta\) Plaques

\(^{99m}\text{Tc}\) (\(T_{1/2} = 6.01\) h, 141 keV) has become the most commonly used radionuclide in diagnostics for SPECT, because it is readily produced by an \(^{99}\text{Mo}/^{99m}\text{Tc}\) generator, the medium 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
Table 3: Biodistribution of radioactivity after injection of $^{99m}$Tc-labeled benzofuran derivatives in normal mice$^a$.

| Organ          | Time after injection (min) 2 | 10 | 30 | 60 |
|----------------|-----------------------------|----|----|----|
|                | $^{99m}$Tc-BAT-BF (27)      |    |    |    |
| 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 (28)     |    |    |    |
| 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         | 7.39 (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 5 mice. Expressed as % injected dose per gram. $^b$Expressed as % injected dose per organ.

Figure 11: Chemical structure of $^{99m}$Tc complexes based on the benzofuran scaffold for imaging of Aβ plaques.

Han and co-workers described a positively charged $^{99}$Tc-complex of Congo red (20) which binds to Aβ aggregates in vitro [50]. The basic structure of this complex is the Congo red backbone in which the biphenyl moiety is replaced by a bipyridyl moiety capable of complexing Tc in the presence of tert-butylisonitrile as a coligand. Although these Tc complexes showed high affinity for Aβ aggregates in vitro, they have not been tested in vivo. Dezutter and co-workers reported a $^{99m}$Tc-labeled conjugate of Congo Red with a monoamido-monoaminedithiol (MAMA) chelating ligand [51]. However, brain uptake of this $^{99m}$Tc-labeled Congo Red derivative (21) was minimal, probably because of its large size and ionized character at physiological pH. Serdons and co-workers reported the synthesis of a neutral $^{99m}$Tc-labeled derivative of thioflavin-T (22), namely a benzothiazole derivative conjugated with a bisamine-bisthiol (BAT) ligand, and its biological characterization [52]. It was demonstrated...
that the $^{99m}$Tc-labeled thioflavin-T derivative binds \textit{in vitro} to A\textbeta plaques. Despite its high lipophilicity and neutral character, the $^{99m}$Tc complex did not cross the blood-brain barrier to a sufficient degree and thus is not useful for the detection of AD \textit{in vivo}. Recently, Chen et al. reported that the $^{99m}$Tc-labeled thioflavin T using MAMA as a chelation ligand (23) demonstrated the binding to A\textbeta aggregates in sections of brain tissue from transgenic mice and AD patients [53]. In addition, 23 can penetrate the blood-brain barrier with high initial brain uptake and moderate washout. These results are encouraging for further exploration of their derivatives as imaging agents for A\textbeta plaques in the brain.

As described above, several $^{99m}$Tc-labeled imaging probes have been developed (Figure 9) [50–55], but no clinical study of them has been reported. While these $^{99m}$Tc complexes showed high affinity for A\textbeta aggregates or A\textbeta plaques \textit{in vitro}, they suffered the same unfavorable \textit{in vivo} pharmacokinetics in normal mice, that is, a slow washout. Therefore, to make them promising probes for imaging A\textbeta plaques in the brain, additional molecular modifications to improve their pharmacokinetics \textit{in vivo} are required.

Recently, we have developed several $^{99m}$Tc complexes based on flavone, chalcone, aurone, and benzofuran derivatives with monoamine-monoamide dithiol (MAMA) and bis-amino-bis-thiol (BAT) as chelation ligands (Figures 10 and 11). MAMA and BAT were selected taking into consideration the permeability of the blood-brain barrier, because they form an electrically neutral complex with $^{99m}$Tc [56]. We then evaluated their biological potential as probes by testing their affinity for A\textbeta aggregates and A\textbeta plaques in sections of brain tissue from Tg2576 mice and their uptake in and clearance from the brain in biodistribution experiments using normal mice.

Initially, four $^{99m}$Tc-labeled chalcone derivatives and their corresponding rhenium analogues were tested as potential probes for imaging A\textbeta plaques (Figure 10) [57]. The chalcones showed higher affinity for A\textbeta(1–42) aggregates than did $^{99m}$Tc complexes and, in sections of brain tissue from an animal model of AD, the four Re-chalcones intensely stained A\textbeta plaques. In biodistribution experiments using normal mice, $^{99m}$Tc-BAT-chalcone (24) displayed high uptake in the brain (1.48%ID/g) at 2 min after injection. The radioactivity washed out from the brain rapidly (0.17%ID/g at 60 min), a highly desirable feature for an imaging agent. Although potential existence of \textit{cis}- and \textit{anti}-isomers was expected, one single isomer was isolated in the preparation of 24, 25, and 26. The chemical identities of 24, 25, and 26 were confirmed by NMR and MS, but their absolute configurations have not yet been determined by X-ray crystallography.

As for $^{99m}$Tc complexes based on benzofuran, we evaluated binding affinity using Re-BAT-BF and Re-MAMA-BF, analogs of $^{99m}$Tc-BAT-BF (27) and $^{99m}$Tc-MAMA-BF (28), respectively. Both ligands inhibited the binding of $^{125}$IIMPY to A\textbeta(1–42) aggregates in a dose-dependent manner, indicating an affinity for A\textbeta aggregates (Figure 12). Their $K_i$ values were 11.5 and 24.4 nM, respectively, suggesting that Re-BAT-BF displayed higher affinity than Re-MAMA-BF. Next, the affinity of $^{99m}$Tc-BAT-BF (27) for A\textbeta plaques was investigated \textit{in vitro} using sections of Tg2576 mouse brain (Figure 13). Furthermore, the radioactivity of $^{99m}$Tc-BAT-BF (27) corresponded with the areas of staining with thioflavin S, a dye commonly used for A\textbeta plaques. In contrast, normal mouse brain displayed no detectable accumulation of $^{99m}$Tc-BAT-BF (27). The results suggest that $^{99m}$Tc-BAT-BF (27) binds to A\textbeta plaques in the mouse brain in addition to synthetic A\textbeta aggregates.

The biodistribution of $^{99m}$Tc-BAT-BF (27) and $^{99m}$Tc-MAMA-BF (28) was examined in normal mice (Table 3). $^{99m}$Tc-BAT-BF (27) showed greater uptake (1.34%ID/g) than $^{99m}$Tc-MAMA-BF (28) (0.74%ID/g) at 2 min after injection. The uptake of $^{99m}$Tc-BAT-BF (27) peaked at 10 min after injection, reaching 1.37%ID/g, sufficient uptake for A\textbeta imaging, and 60% of the radioactivity had been washed out from the brain by 60 min after injection. The uptake of $^{99m}$Tc-MAMA-BF (28) 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 (27) throughout the time course, which is unsuitable for imaging \textit{in vivo}. The combination of good affinity for A\textbeta plaques, uptake,
and clearance makes $^{99m}$Tc-BAT-BF (27) a promising probe for the detection of $\beta$$\eta$ 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 A$\beta$ plaques in the brain, although there are some difficulties associated with the large size of $^{99m}$Tc complex in the molecular design of $^{99m}$Tc-labeled A$\beta$ imaging probes to enhance the penetration of blood-brain barrier.

4. Conclusion

Many PET probes targeting A$\beta$ plaques in the brain have been tested clinically and demonstrated potential utility. Unfortunately, the short half-life ($^{11}$C; 20 min, $^{18}$F; 110 min) of $^{11}$C- or $^{18}$F-labeled probes except $^{18}$F-FDG limits their use at major academic PET facilities with on-site cyclotrons and sophisticated radiochemistry laboratories. On the other hand, many more hospitals have the capacity to perform SPECT. A$\beta$ imaging probes labeled with SPECT isotopes especially the inexpensive and readily available $^{99m}$Tc will have more widespread clinical applicability especially in developing countries that cannot afford expensive cyclotron and PET scanners. The development of novel $^{123}$I- or $^{99m}$Tc-labeled A$\beta$ imaging probes may lead to simple and convenient SPECT imaging methods for detecting and eventually quantifying A$\beta$ plaques in living brain tissue.

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References

[1] D. J. Selkoe, “Alzheimer’s disease: genes, proteins, and therapy,” Physiological Reviews, vol. 81, no. 2, pp. 741–766, 2001.
[2] J. A. Hardy and G. A. Higgins, “Alzheimer’s disease: the amyloid cascade hypothesis,” Science, vol. 256, no. 5054, pp. 184–185, 1992.
[3] C. A. Mathis, Y. Wang, and W. E. Klunk, “Imaging $\beta$-amyloid plaques and neurofibrillary tangles in the aging human brain,” Current Pharmaceutical Design, vol. 10, no. 13, pp. 1469–1492, 2004.
[4] A. Nordberg, “PET imaging of amyloid in Alzheimer’s disease,” Lancet Neurology, vol. 3, no. 9, pp. 519–527, 2004.
[5] M. Ono, “Development of positron-emission tomography/single-photon emission computed tomography imaging probes for in vivo detection of $\beta$-amyloid plaques in Alzheimer’s brains,” Chemical and Pharmaceutical Bulletin, vol. 57, no. 10, pp. 1029–1039, 2009.
[6] N. P. L. G. Verhoeff, A. A. Wilson, S. Takeshita et al., “In vivo imaging of Alzheimer disease $\beta$-amyloid with $^{11}$C-SB-13 PET,” American Journal of Geriatric Psychiatry, vol. 12, no. 6, pp. 584–595, 2004.
[7] H. F. Kung, S. R. Choi, W. Qu, W. Zhang, and D. Skovronsky, “$^{18}$F stilbenes and styrlypyridines for PET imaging of A$\beta$ plaques in Alzheimer’s disease: a miniperspective,” Journal of Medicinal Chemistry, vol. 53, no. 3, pp. 933–941, 2010.
[8] C. A. Mathis, Y. Wang, D. P. Holt, G. F. Huang, M. L. Debnath, and W. E. Klunk, “Synthesis and evaluation of $^{11}$C-labeled 6-substituted 2-arylbenzothiazoles as amyloid imaging agents,” Journal of Medicinal Chemistry, vol. 46, no. 13, pp. 2740–2754, 2003.
[9] W. E. Kung, H. Engler, A. Nordberg et al., “Imaging brain amyloid in Alzheimer’s disease with Pittsburgh Compound-B,” Annals of Neurology, vol. 55, no. 3, pp. 306–319, 2004.
[10] N. M. Scheinin, S. Aalto, J. Koikkalainen et al., “Follow-up of $^{11}$C[PIB uptake and brain volume in patients with Alzheimer disease and controls,” Neurology, vol. 73, no. 15, pp. 1186–1192, 2009.
[11] M. Ono, A. Wilson, J. Nobrega et al., “$^{11}$C-labeled stilbene derivatives as A$\beta$-aggregate-specific PET imaging agents for Alzheimer’s disease,” Nuclear Medicine and Biology, vol. 30, no. 6, pp. 565–571, 2003.
[12] Y. Kudo, N. Okamura, S. Furumoto et al., “2-(2-[2-dimethylaminothiazol-5-yl][ethenyl]-6-(2-[fluoroethoxy]benzoxazole: a novel PET agent for in vivo detection of dense amyloid plaques in Alzheimer’s disease patients,” Journal of Nuclear Medicine, vol. 48, no. 4, pp. 553–561, 2007.
[13] A. E. Johnson, F. Jeppsson, J. Sandell et al., “AZD2184: a radioligand for sensitive detection of $\beta$-amyloid deposits,” Journal of Neurochemistry, vol. 108, no. 5, pp. 1177–1186, 2009.
[14] E. D. Agdeppa, V. Kepe, J. Liu et al., “Binding characteristics of radiofluorinated 6-dialkylamino-2-naphthylethylidene derivatives as positron emission tomography imaging probes for beta-amyloid plaques in Alzheimer’s disease,” The Journal of Neuroscience, vol. 21, no. 24, p. RC189, 2001.
[15] K. Shoghi-Jadid, G. W. Small, E. D. Agdeppa et al., “Localization of neurofibrillary tangles and beta-amyloid plaques in the brains of living patients with Alzheimer disease,” American Journal of Geriatric Psychiatry, vol. 10, no. 1, pp. 24–35, 2002.
[16] G. W. Small, V. Kepe, L. M. Ercoli et al., “PET of brain amyloid and tau in mild cognitive impairment,” New England Journal of Medicine, vol. 355, no. 25, pp. 2652–2663, 2006.
[17] W. Zhang, S. Oya, M. P. Kung, C. Hou, D. L. Maier, and H. F. Kung, “F-18 Polyethylene glycol stilbene as PET imaging agents targeting A$\beta$ aggregates in the brain,” Nuclear Medicine and Biology, vol. 32, no. 8, pp. 799–809, 2005.
[18] C. C. Rowe, U. Ackerman, W. Browne et al., “Imaging of amyloid $\beta$ in Alzheimer’s disease with $^{18}$F-BAY94-9172, a novel PET tracer: proof of mechanism,” The Lancet Neurology, vol. 7, no. 2, pp. 129–135, 2008.
[19] W. Zhang, M. P. Kung, S. Oya, C. Hou, and H. F. Kung, “$^{18}$F-labeled styrlypyridines as PET agents for amyloid plaque imaging,” Nuclear Medicine and Biology, vol. 34, no. 1, pp. 89–97, 2007.
[20] S. R. Choi, G. Golding, Z. Zhuang et al., “Preclinical properties of F-AV-45: A PET agent for A$\beta$ plaques in the brain,” Journal of Nuclear Medicine, vol. 50, no. 11, pp. 1887–1894, 2009.
[21] D. F. Wong, P. B. Rosenberg, Y. Zhou et al., “In vivo imaging of amyloid deposition in Alzheimer disease using the radioligand 18F-F-AV-45 (flubetapir F 18),” Journal of Nuclear Medicine, vol. 51, no. 6, pp. 913–920, 2010.

[22] M. Koole, D. M. Lewis, C. Buckley et al., “Whole-body biodistribution and radiation dosimetry of 18F-F-GE067: a radioligand for in vivo brain amyloid imaging,” Journal of Nuclear Medicine, vol. 50, no. 5, pp. 818–822, 2009.

[23] M. Ono, M. Haratake, M. Nakayama et al., “Synthesis and biological evaluation of (E)-3-styrylpyridine derivatives as amyloid imaging agents for Alzheimer’s disease,” Nuclear Medicine and Biology, vol. 32, no. 4, pp. 329–335, 2005.

[24] W. Qu, M. P. Kung, C. Hou, T. E. Benedum, and H. F. Kung, “Novel styrylpyridines as probes for SPECT imaging of amyloid plaques,” Journal of Medicinal Chemistry, vol. 50, no. 9, pp. 2157–2165, 2007.

[25] W. Qu, M. P. Kung, C. Hou, L. W. Jin, and H. F. Kung, “Radiiodinated azadiphenylactenyles as potential SPECT imaging agents for β-amyloid plaque detection,” Bioorganic and Medicinal Chemistry Letters, vol. 17, no. 13, pp. 3581–3584, 2007.

[26] M. P. Kung, C. Hou, Z. P. Zhuang et al., “IMPY: an improved thioflavin-T derivative for in vivo labeling of β-amyloid plaques,” Brain Research, vol. 956, no. 2, pp. 202–210, 2002.

[27] Z. P. Zhuang, M. P. Kung, A. Wilson et al., “Structure-activity relationship of imidazo[1,2-a]pyridines as ligands for detecting β-amyloid plaques in the brain,” Journal of Medicinal Chemistry, vol. 46, no. 2, pp. 237–243, 2003.

[28] M. P. Kung, C. Hou, Z. P. Zhuang, D. Skovronsky, and H. F. Kung, “Binding of two potential imaging agents targeting amyloid plaques in postmortem brain tissues of patients with Alzheimer’s disease,” Brain Research, vol. 1025, no. 1-2, pp. 98–105, 2004.

[29] Z. P. Zhuang, M. P. Kung, C. Hou et al., “Radiiodinated styrylbenzenes and thioflavins as probes for amyloid aggregates,” Journal of Medicinal Chemistry, vol. 44, no. 12, pp. 1905–1914, 2001.

[30] Z. P. Zhuang, M. P. Kung, C. Hou et al., “IBOX(2-(4‘-dimethylaminophenyl)-6-iodobenzoxazole): a ligand for imaging amyloid plaques in the brain,” Nuclear Medicine and Biology, vol. 28, no. 8, pp. 887–894, 2001.

[31] M. Ono, M. P. Kung, C. Hou, and H. F. Kung, “Benzofuran derivatives as βAmyloid-aggregate-specific imaging agents for Alzheimer’s disease,” Nuclear Medicine and Biology, vol. 29, no. 6, pp. 633–642, 2002.

[32] H. Watanabe, M. Ono, M. Haratake, N. Kobashi, H. Saji, and M. Nakayama, “Synthesis and characterization of novel phenylindoles as potential probes for imaging of β-amyloid plaques in the brain,” Bioorganic and Medicinal Chemistry, vol. 18, no. 13, pp. 4740–4746, 2010.

[33] M. P. Kung, C. Hou, Z. P. Zhuang, A. J. Cross, D. L. Maier, and H. F. Kung, “Characterization of IMPY as a potential imaging agent for β-amyloid plaques in double transgenic PSAPP mice,” European Journal of Nuclear Medicine and Molecular Imaging, vol. 31, no. 8, pp. 1136–1145, 2004.

[34] A. B. Newberg, N. A. Wintering, C. M. Clark et al., “Use of 123I IMPY SPECT to differentiate Alzheimer’s disease from controls,” The Journal of Nuclear Medicine, vol. 47, supplement 1, p. 78P, 2006.

[35] A. B. Newberg, N. A. Wintering, K. Plössl et al., “Safety, biodistribution, and dosimetry of 123I-IMPY: a novel amyloid plaque-imagining agent for the diagnosis of Alzheimer’s disease,” Journal of Nuclear Medicine, vol. 47, no. 5, pp. 748–754, 2006.

[36] K. Ono, Y. Yoshiike, A. Takashima, K. Hasegawa, H. Naiki, and M. Yamada, “Potent anti-amyloidogenic and fibril-stabilizing effects of polyphenols in vitro: implications for the prevention and therapeutics of Alzheimer’s disease,” Journal of Neurochemistry, vol. 87, no. 1, pp. 172–181, 2003.

[37] M. Ono, N. Yoshida, K. Ishibashi et al., “Radiiodinated flavones for in vivo imaging of β-amyloid plaques in the brain,” Journal of Medicinal Chemistry, vol. 48, no. 23, pp. 7253–7260, 2005.

[38] T. Iwatsubo, A. Odaka, N. Suzuki, H. Mizusawa, N. Nukina, and Y. Ihara, “Visualization of Aβ42(43) and Aβ40 in senile plaques with end-specific Aβ monoclonals: evidence that an initially deposited species is Aβ42(43),” Neuron, vol. 13, no. 1, pp. 45–53, 1994.

[39] J. C. Morris, M. Storandt, D. W. McKeel et al., “Cerebral amyloid deposition and diffuse plaques in “normal” aging: evidence for presymptomatic and very mild Alzheimer’s disease,” Neurology, vol. 46, no. 3, pp. 707–719, 1996.

[40] H. F. Kung, C. W. Lee, Z. P. Zhuang, M. P. Kung, C. Hou, and K. Plössl, “Novel stilbenes as probes for amyloid plaques,” Journal of the American Chemical Society, vol. 123, no. 50, pp. 12740–12741, 2001.

[41] M. Ono, M. Haratake, H. Mori, and M. Nakayama, “Novel chalcones as probes for in vivo imaging of β-amyloid plaques in Alzheimer’s brains,” Bioorganic and Medicinal Chemistry, vol. 15, no. 21, pp. 6802–6809, 2007.

[42] M. Ono, M. Hori, M. Haratake, T. Tomiyama, H. Mori, and M. Nakayama, “Structure-activity relationship of chalcones and related derivatives as ligands for detecting β-amyloid plaques in the brain,” Bioorganic and Medicinal Chemistry, vol. 15, no. 19, pp. 6388–6396, 2007.

[43] M. Ono, M. Haratake, H. Mori, and M. Nakayama, “Novel chalcones as probes for in vivo imaging of β-amyloid plaques in Alzheimer’s brains,” Bioorganic and Medicinal Chemistry, vol. 15, no. 21, pp. 6802–6809, 2007.

[44] M. Ono, Y. Maya, M. Haratake, K. Ito, H. Mori, and M. Nakayama, “Aurones serve as probes of β-amyloid plaques in Alzheimer’s disease,” Biochemical and Biophysical Research Communications, vol. 361, no. 1, pp. 116–121, 2007.

[45] Y. Maya, M. Ono, H. Watanabe, M. Haratake, H. Saji, and M. Nakayama, “Novel radioiodinated aurones as probes for SPECT imaging of β-amyloid plaques in the brain,” Bioconjugate Chemistry, vol. 20, no. 1, pp. 95–101, 2009.

[46] W. Qu, M. P. Kung, C. Hou, S. Oya, and H. F. Kung, “Quick assembly of 1,4-diphenylthiazoles as probes targeting β-amyloid aggregates in Alzheimer’s disease,” Journal of Medicinal Chemistry, vol. 50, no. 14, pp. 3380–3387, 2007.

[47] R. Chandra, M. P. Kung, and H. F. Kung, “Design, synthesis, and structure-activity relationship of novel thiophene derivatives for β-amyloid plaque imaging,” Bioorganic and Medicinal Chemistry Letters, vol. 16, no. 5, pp. 1350–1352, 2006.

[48] M. Ono, M. Haratake, H. Saji, and M. Nakayama, “Development of novel β-amyloid probes based on 3,5-diphenyl-1,2,4-oxadiazole,” Bioorganic and Medicinal Chemistry, vol. 16, no. 14, pp. 6867–6872, 2008.

[49] H. Watanabe, M. Ono, R. Ikeoka, M. Haratake, H. Saji, and M. Nakayama, “Synthesis and biological evaluation of radiiodinated 2,5-diphenyl-1,3,4-oxadiazoles for detecting β-amyloid plaques in the brain,” Bioorganic and Medicinal Chemistry, vol. 17, no. 17, pp. 6402–6406, 2009.

[50] H. Han, C. G. Cho, and P. T. Lansbury Jr., “Technetium complexes for the quantitation of brain amyloid,” Journal of the American Chemical Society, vol. 118, no. 18, pp. 4506–4507, 1996.
[51] N. A. Dezutter, R. J. Dom, T. J. De Groot, G. M. Bormans, and A. M. Verbruggen, “\(^{99m}\text{Tc}\)-MAMA-chrysamine G, a probe for beta-amyloid protein of Alzheimer’s disease,” *European Journal of Nuclear Medicine*, vol. 26, no. 11, pp. 1392–1399, 1999.

[52] K. Serdons, T. Verduyckt, J. Cleynhens et al., “Synthesis and evaluation of a \(^{99m}\text{Tc}\)-BAT-phenylbenzothiazole conjugate as a potential in vivo tracer for visualization of amyloid \(\beta\),” *Bioorganic and Medicinal Chemistry Letters*, vol. 17, no. 22, pp. 6086–6090, 2007.

[53] X. Chen, P. Yu, L. Zhang, and B. Liu, “Synthesis and biological evaluation of \(^{99m}\text{Tc}\), Re-monoamine-monoamide conjugated to 2-(4-aminophenyl)benzothiazole as potential probes for \(\beta\)-amyloid plaques in the brain,” *Bioorganic and Medicinal Chemistry Letters*, vol. 18, no. 4, pp. 1442–1445, 2008.

[54] Z. P. Zhuang, M. P. Kung, C. Hou, K. Ploessl, and H. F. Kung, “Biphenyls labeled with technetium 99m for imaging \(\beta\)-amyloid plaques in the brain,” *Nuclear Medicine and Biology*, vol. 32, no. 2, pp. 171–184, 2005.

[55] K. S. Lin, M. L. Debnath, C. A. Mathis, and W. E. Klunk, “Synthesis and \(\beta\)-amyloid binding properties of rhenium 2-phenylbenzothiazoles,” *Bioorganic and Medicinal Chemistry Letters*, vol. 19, no. 8, pp. 2258–2262, 2009.

[56] S. Oya, K. Ploessl, M. P. Kung, D. A. Stevenson, and H. F. Kung, “Small and neutral TC(V)O BAT, bisaminoethanethiol (NS) complexes for developing new brain imaging agents,” *Nuclear Medicine and Biology*, vol. 25, no. 2, pp. 135–140, 1998.

[57] M. Ono, R. Ikeoka, H. Watanabe et al., “Synthesis and evaluation of novel chalcone derivatives with \(^{99m}\text{Tc}/\text{Re}\) complexes as potential probes for detection of \(\beta\)-amyloid plaques,” *ACS Chemical Neuroscience*, vol. 1, no. 9, pp. 598–607, 2010.