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Research paper

(*R*)- and (*S*)-^{18}F-labeled 2-arylbenzofurans with improved pharmacokinetics as β-amyloid imaging probes

Jia Song [a], Xiaoyang Zhang [a], Yunling Zhao [a], Hui Yang [b], Jinming Zhang [b, **], Xiaojun Zhang [b], Jiapei Dai [c], Mengchao Cui [a, *]

[a] Key Laboratory of Radiopharmaceuticals, Ministry of Education, College of Chemistry, Beijing Normal University, Beijing 100875, PR China
[b] Department of Nuclear Medicine, Chinese PLA General Hospital, Beijing 100853, PR China
[c] Wuhan Institute for Neuroscience and Neuroengineering, South-Central University for Nationalities, Wuhan 430074, PR China

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ABSTRACT

A new class of optical isomers of 2-arylbenzofuran derivatives were synthesized and evaluated as potential β-amyloid plaques imaging agents. Both lipophilicity and signal-to-noise ratio were significantly improved by adding a chiral hydroxyl group to 1-fluoro-3-(oxidanyl)propan-2-ol side chain. These derivatives displayed moderate to high binding affinity towards A$\beta_{1\text{-}42}$ aggregates. Four tracers possessing potent binding affinity (K_i < 30 nM) were chosen for further investigation. In *in vitro* autoradiography studies, the four selected probes showed effective binding to Aβ plaques in Tg mouse and AD human brain tissue after labeled by ^{18}F. The purified enantiomers displayed apparent discrepancy in biodistribution experiments in normal mice, for (*S*)-enantiomers provided rather faster clearance than (*R*)-enantiomers. All in all, (*S*)-[^{18}F]**17** (K_i = 14.6 nM) with excellent pharmacokinetics ($brain_{2\ min}$ = 8.60% ID/g, $brain_{2\ min}/brain_{60\ min}$ = 14.1) deserves further evaluation.

1. Introduction

Alzheimer's disease (AD), a complex neurodegenerative disease, is becoming an extensive public health issue [1]. Despite continual technical advancement and intensive studies, detailed understanding of the sophisticated mechanism is still unrevealed. Amyloid cascade hypothesis is a prevailing theory in terms of the pathogenesis of AD, on which constant evidences have elucidated that β-amyloid plaques play a critical role in this insidious and lethal neuropathy [2]. Amyloid plaques could be found in the brains of nearly all patients of AD and usually developed a couple of years prior to the onset of dementia [3,4]. What worth mentioning is that molecular imaging techniques have provided extraordinary insight into human brain function. Especially, positron emission tomography (PET) and single photon emission computed tomography (SPECT) have been providing an essential access to the diagnosis and prognosis of AD using radioactive imaging probes [5]. Accordingly, high-affinity Aβ-specific imaging agents are of fundamental importance and there is a dire need of effective tracers for imaging.

Over the previous decades, an array of Aβ-specific neuroimaging radiotracers have been generated from the scaffolds of Congo red (CR) and Thioflavin-T (Th-T) [6,7]. Among the substantial number of reported amyloid imaging agents, [^{11}C]PIB, the lipophilic analog of Th-T was one of the most famous, which has been extensively studied in humans [8,9]. Unfortunately, the clinical application of compounds labeled by ^{11}C has been limited by its short half-life ($t_{1/2}$ = 20 min). Then an increasing tendency of studying other radionuclides such as ^{18}F ($t_{1/2}$ = 110 min) with a longer half-life is underway to overcome that deficiency. The study of ^{18}F-labeled Aβ probes indeed pushed the development of radiopharmaceutical clinical utilize forward as expected. [^{18}F]AV-45 and other two ^{18}F-labeled radiotracers ([^{18}F]GE-067 and [^{18}F]BAY94-9172) have been approved by the U.S. Food and Drug Administration for clinical evaluation of patients suspected with AD [10-13]. However, there are still some results unsatisfied due to their high lipophilicity and slow clearance rate from the normal brain, which may lead to lower signal-to-noise ratio and influence the accuracy of PET scans.

On the basis of the previous research that a fluoro-polyethyleneglycol (FPEG) group was employed on stilbene scaffold, Kung *et al* introduced an additional hydroxyl group on the

* Corresponding author.
** Corresponding author.
E-mail addresses: zhangjm301@yahoo.com.cn (J. Zhang), cmc@bnu.edu.cn (M. Cui).

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fluoroalkyl side chain at the same position. According to obtained results, the additional hydroxyl group did reduce lipophilicity of [^{18}F]FMAPO (log $P = 2.95$) to a certain extent and further improve the initial brain uptake and washout rate (9.75% ID/g at 2 min and 0.72% ID/g at 60 min) by a comparison with [^{18}F]AV-45 (7.33% ID/g at 2 min and 1.80% ID/g at 60 min) [10,14]. However, the existence of a chiral center in the molecule may cause complicated results in living body. Then they abandoned it and turned to [^{18}F]AV-45. Later, Cheng *et al* reported a series of compounds with the similar FPEG group applied to pyridylbenzofuran and phenylbenzofuran scaffold. But the experiment results showed that the pharmacokinetic properties of these compounds, containing initial brain uptake (5.66% ID/g for [^{18}F]FPHBF-1 and 5.16% ID/g for [^{18}F]FPYBF-1 at 2 min) and brain$_{2\ min}$/brain$_{60\ min}$ ratio (1.0 and 2.1, respectively), were not as well as [^{18}F]AV-45. At the same time, they tested another benzofuran derivative possessing the same side chain as [^{18}F]FMAPO. However, the low radiochemical yield (<0.1%) made it difficult to conduct further experiments [15,16]. (Fig. 1)

Very recently, we have reported a series of enantiopure ^{18}F-labeled 2-arylbenzothiazole and 2-arylbenzoxazole derivatives with a chiral 1-fluoro-3-(oxidanyl)propan-2-ol side chain as Aβ tracers for PET. Compared with FPEG modified 2-arylbenzoheterocyclic structure, both lipophilicity and signal-to-noise ratio were well improved. Furthermore, a significant difference on the brain kinetics was observed between the (*R*) and (*S*) isomers [17]. Based on these observations, herein we applied this chiral side chain to the 2-arylbenzofuran scaffold to further improve the brain kinetics.

2. Results and discussion

2.1. Chemistry

The synthesis of fluorinated benzofuran derivatives is outlined in Scheme 1. The starting compounds **1**–**8** were prepared through the methods reported previously [18-20]. The desired epoxide compounds (*R*)/(*S*)-**9**-**16** were obtained by Williamson synthesis between **1**-**8** and enantiopure (*R*)-(−)- and (*S*)-(+)-epichlorohydrin in yields of 20.5–56.3%. In this way, we avoided complicated chiral separation procedure such as semi-preparative chiral supercritical fluid chromatography (SFC) [21]. And then, the epoxide groups of (*R*)/(*S*)-**9**-**16** were opened regioselectively using tetrabutylammonium fluoride (TBAF) in toluene which afforded the fluorinated derivatives (*R*)/(*S*)-**17**-**24** in yields of 34.6–81.9%. Enantiomeric purities of (*R*)/(*S*)-**17** and **18** reached 99% by chiral HPLC analysis. Chiral HPLC chromatograms are shown in Fig. S2 in supporting information.

The synthesis of tetrahydropyran (THP) protected tosylate precursors is outlined in Scheme 2. (*R*)/(*S*)-**25** and **31** were prepared by coupling of **1** and **2** with (*R*)-(+)- or (*S*)-(−)-3-chloropropane-1,2-diol in EtOH in yields of 40.2–87.7%. For the *N*-monomethylated (*R*)/(*S*)-**25**, two protection steps were successively applied to the two hydroxyl groups and the monomethyl amino group. Then the *tert*-butyldimethylsilyl protection group was deprotected by TBAF. The terminal hydroxyl group of diols in (*R*)/(*S*)-**28** and (*R*)/(*S*)-**31** was regioselectively monotosylated with *p*-tosyl chloride (TsCl) to afford (*R*)/(*S*)-**29** and (*R*)/(*S*)-**32** in yields of 17.6–44.7%. Finally, the tosylate precursors (*R*)/(*S*)-**30** and **33** were achieved by (*R*)/(*S*)-**29** and **32** with its remanent hydroxyl group protected with THP.

2.2. In vitro binding assay using Aβ aggregates

The binding affinity of these fluorinated (*R*)/(*S*)-2-arylbenzofuran derivatives ((*R*)/(*S*)-**17**-**24**) for A$\beta_{1\text{-}42}$ aggregates were quantitatively evaluated by competitive binding assay using [^{125}I]4-(6-iodoimidazo [1,2-a]pyridin-2-yl)-*N*,*N*-dimethylaniline ([^{125}I]IMPY) as radio-ligand. As shown in Table 1, most of these derivatives displayed high affinity to A$\beta_{1\text{-}42}$ aggregates, of which (*R*)/(*S*)-**18** displayed the highest affinity with K_i values of 6.7 and 9.7 nM better than IMPY (K_i = 17.3 nM) under the same assay conditions. The position of substituent group on the benzofuran moiety had an interesting impact on binding affinity. Compared with the ligands with substituent group at 6-position, the 5-position substituted ligands exhibited slightly higher binding affinity, and this phenomenon was consistent with the results for 2-phenylbenzoxazole scaffold [17]. As observed in most other scaffolds studied so far, the ligands with *N*,*N*-dimethylamino group (*R*)/(*S*)-**18**, **20**, **22**, **24** displayed higher binding affinity than ligands with *N*-monomethylamino group (*R*)/(*S*)-**17**, **19**, **21**, **23** [15,22,23]. Besides, the affinity decreased when an electronegative nitrogen atom was introduced into the aminophenyl group. Thus, ligand (*S*)-**23** with three negative factors, 6-substituent side chain, *N*-monomethylamino group and pyridyl substitution, displayed the lowest bind affinity (K_i = 691.3 nM). The existence of chiral center didn't affect the binding affinity of these benzofuran derivatives significantly, and the K_i values of (*R*)/(*S*)-enantiomers displayed inconspicuous difference unlike the benzoxazole and benzothiazole scaffolds. Especially, ligands (*R*)/(*S*)-**17**, **18** exhibited potent binding affinity to A$\beta_{1\text{-}42}$ aggregates with K_i values lower than 30 nM were selected for ^{18}F labeling and further biological studies.

2.3. Radiochemistry

The ^{18}F-labeled ligands were prepared by an one-pot two-step method, nucleophilic substitution reaction followed by acid hydrolysis of the THP or Boc protection group. As shown in Scheme 3, the tosylate precursors were first reacted with [^{18}F]fluoride/Kryptofix$_{222}$/K_2CO_3 in anhydrous acetonitrile at 100 °C for 8 min. Then

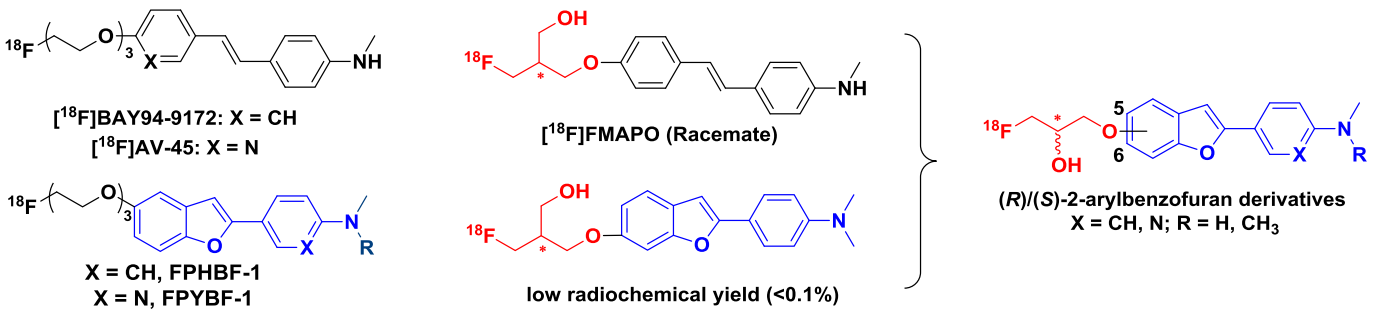

Fig. 1. Design concept of the new (*R*)/(*S*)-^{18}F-labeled 2-arylbenzofuran derivatives.