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# Synthesis and biological evaluation of 2-(3,4-dimethoxyphenyl)-6-(2-[$^{18}$F]fluoroethoxy)benzothiazole ([$^{18}$F]FEDBT) for PET imaging of breast cancer

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ABSTRACT

Given the ever-present demand for improved PET radiotracer in oncology imaging, we have synthesized 2-(3,4-dimethoxyphenyl)-6-(2-[$^{18}$F]fluoroethoxy)benzothiazole ([$^{18}$F]FEDBT), a fluorine-18-containing fluoroethylated benzothiazole to explore its utility as a PET imaging tracer. [$^{18}$F]FEDBT was prepared via kryptofix-mediated nucleophilic substitution of the tosyl group precursor. Fractionated ethanol-based solid-phase (SPE cartridge-based) purification afforded [$^{18}$F]FEDBT in 60% radiochemical yield (EOB), with radiochemical purity in excess of 98% and the specific activity was 35 GBq/μmol. The radiotracer displayed clearly higher cellular uptake ratio in various breast cancer cell lines MCF7, MDA-MB-468 and MDA-MB-231. However, both biodistribution and microPET studies have showed an higher abdominal accumulation of [$^{18}$F]FEDMBT and the tumor/muscle ratio of 1.8 was observed in the MDA-MB-231 xenograft tumors mice model. Further the lipophilic improvement is needed for the reducement of hepatobilliary accumulation and to promote the tumor uptake for PET imaging of breast cancer.



Breast cancer is the most prevalent malignancy and one of the major causes of cancer death in women worldwide. Approximately 15–20% of all breast cancer cases can be classified as triple-negative breast cancer (TNBC), characterized by the absence of gene expression for estrogen receptors (ERs), progesterone receptors (PRs) and human epidermal growth factor-2 (HER-2).[1–3] Clinical studies reveal that TNBC patients show significantly higher rates of recurrence at distant sites and poor prognosis.[4] Therefore, early detection and molecular typing of breast cancer is essential for improving efficacy of therapeutic interventions and increasing survival rates.[5] Mammography is a major tool for screening and early detection of breast cancer, however, its sensitivity is limited and frequent false positive diagnoses are a problem. Compared to standard X-ray mammography, positron emission tomography (PET), especially hybrid PET-CT imaging, can be used not only to detect cancer, but also to stage it, determine metabolic properties and biological status of the tumor, as well as help with the therapeutic response evaluation.[6–8]

Recently, versatile 2-arylbezothiazole scaffold based compounds with high potential for diagnostic and therapeutic applications have been developed (Fig. 1).[9,10] Carbon-11 labeled benzothiazole aniline (BTA) derivative, known as [$^{11}$C]PIB or Pittsburgh compound-B, demonstrated good binding properties towards Aβ plaques and excellent pharmacokinetics for imaging brain amyloid in Alzheimer's disease.[11] The corresponding $^{18}$F-labeled analogue known as [$^{18}$F]flutemetamol (Vizamyl)[12] with similar diagnostic properties and benefits of longer half-life of fluorine-18 was approved for clinical PET application by FDA in 2013. As a matter of interest, a series of compounds based on 2-arylbezothiazole described by Westwell et al. were found to inhibit tyrosine kinase.[13,14] The lead compound of the library – 5-fluoro-2-(3,4-dimethoxyphenyl)benzothiazole (PMX-610) has demonstrated very potent ($GI_{50}$ < 0.1 nM for MCF-7 and MDA-468 cell

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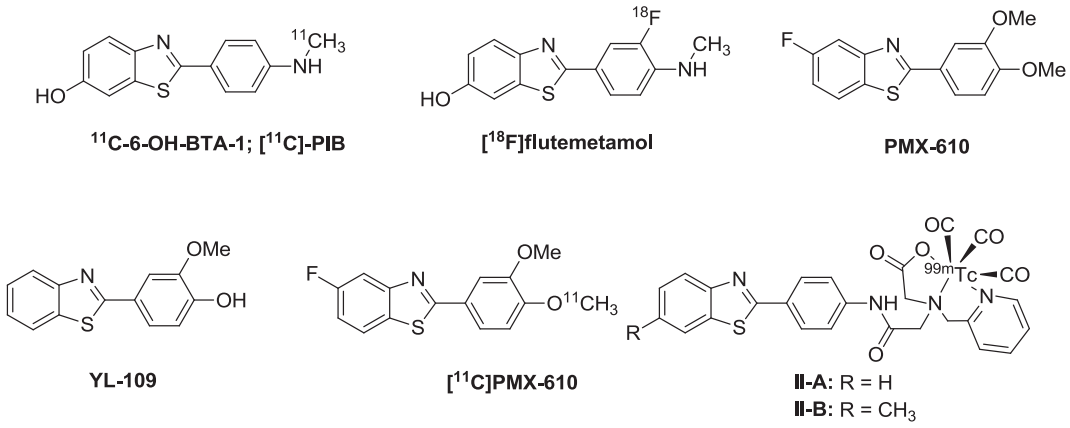
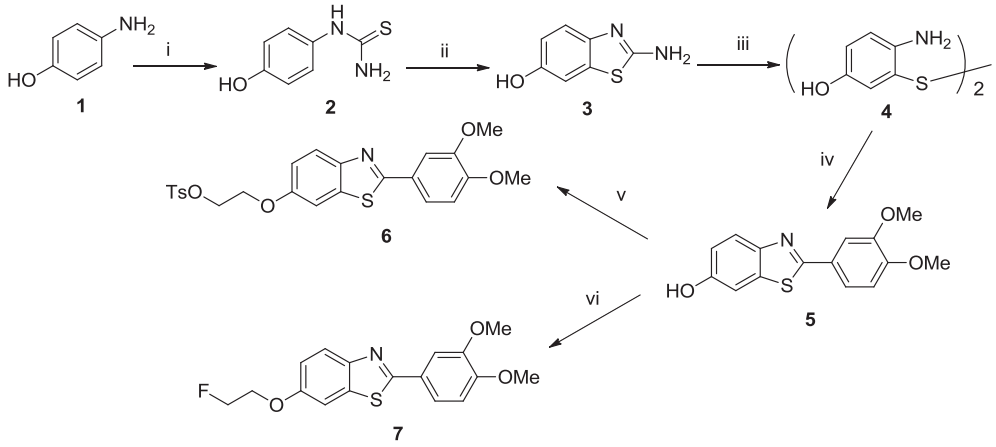



**Fig. 1.** Chemical structures of the diagnostic or therapeutic 2-arylbenzothiazoles.




**Scheme 1.** Synthesis of the labeling precursor **6** and authentic standard **7**: (i) 4-hydroxyaniline, KSCN, HCl, reflux 4 h; (ii) $Br_2$, chloroform, reflux 4 h; (iii) KOH, $H_2O$, ethylene glycol, reflux 12 h; (iv) 3,4-dimethoxybenzaldehyde, *p*-toluene sulfonic acid, $PPh_3$, Toluene, reflux 15 h; (v) ethylene di(*p*-toluenesulfonate), potassium carbonate, $CH_3CN$, reflux 12 h; (vi) 1-fluoro-2-tosyloxyethane, potassium carbonate, $CH_3CN$, reflux 12 h.

lines) and selective *in vitro* antiproliferative properties for human lung, colon and breast cancer cell lines.[14] Proposed mechanisms are based on the selective high affinity of aryl hydrocarbon Receptor (AhR) which act as both inducers and substrates for P450s 1A1 and 2W1. The PMX-610 forms activated AhR complexes which translocate to the nucleus inducing cypa1a1 gene transcription and expression of P450s 1A1 and 2W1 enzyme. The oxidation products of PMX-610 can form GSH conjugates which may lead to their possible toxicological consequences and result in formation of DNA adducts and ultimately cell death.[15–17] Recently, Hiyoshi and co-workers also found a novel benzothiazole analogue, 2-(4-hydroxy-3-methoxyphenyl)benzothiazole (YL-109), with ability to inhibit TNBC cell growth and induce CHIP (C-terminus of Hsp70-Interacting Protein) transcription through AhR signaling in MDA-MB-231 cells.[18] AhR overexpression and its activation are considered to be markers that predict sensitivity of breast cancer cells to benzothiazole treatment. Therefore, development of new PET imaging probes to evaluate response of tumors to benzothiazole-based drugs is of significant importance. To date, only a handful of such PET and SPECT radiotracers have been reported. The [$^{11}$C]PMX-610 and other carbon-11 labeled 2-arylbezothiazole analogues have been prepared via standard *O*-$^{11}$C-methylation, but their potential as PET imaging agents needs to be further evaluated.[19] The technecium-99m labeled complexes of 2-(4′-aminophenyl)benzothiazole II-A and II-B (Fig. 1) were developed by Pelecanou and co-workers as promising radiotracers for SPECT imaging of breast cancer.[20,21] In biodistribution studies the tumor/muscle ratio of 2.2 for the most active complex II-B was observed using MCF-7 tumor bearing SCID mice, indicating suitability of benzothiazole scaffold for the development of radiopharmaceuticals.

To translate chemotherapeutic compound into diagnostic application in PET imaging, we have taken aim at developing a fluorine-18 labeled derivative of PMX-610, 2-(3,4-dimethoxyphenyl)-6-(2-[$^{18}$F]fluoroethoxy)benzothiazole ([$^{18}$F]FEDBT). Introduction of $^{18}$F-label into alkyl chain can be easily achieved via an $S_N2$ substitution reaction of tosylate in an appropriate precursor with an activated [$^{18}$F]fluoride.

We further report the results of an *in vitro* assessment of [$^{18}$F]FEDBT properties using MCF-7, MDA-MB-468 and MDA-MB-231 breast cancer cell lines as well as results of *in vivo* studies with MDA-MB-231 breast tumor bearing mice evaluating the potential of this benzothiazole derivative as breast cancer PET imaging agent.

The standard compound **7** and precursor **6** were synthesized in five steps following previously published methods with minor modification from the commercially available 4-hydroxyaniline as it is presented on the Scheme 1.[19,22] Briefly, the aryl thiourea **2** was synthesized via condensation of 4-hydroxyaniline and potassium thiocyanate. The 2-amino-6-hydroxybenzothiazole **3** was obtained from the oxidative cyclization of compound **2** with bromine, followed by one-pot reaction of **3** with aqueous potassium