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Synthesis of carbon-11 labeled fluorinated 2-arylbenzothiazoles as novel potential PET cancer imaging agents

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Abstract—Fluorinated 2-arylbenzothiazoles are new potential antitumor drugs, which show potent and selective inhibitory activity against breast, lung, and colon cancer cell lines. Carbon-11 labeled fluorinated 2-arylbenzothiazoles may serve as novel probes for positron emission tomography (PET) to image tyrosine kinase in cancers. The preparation of 4-fluorinated 2-arylbenzothiazoles 4-fluoro-2-(3-benzloxy-4-methoxyphenyl)benzothiazole (**6a**) and 4-fluoro-2-(3,4-dimethoxyphenyl)benzothiazole (**6b**) was achieved by a modification of Jacobson thioanilide radical cyclization chemistry. Hydrogenolytic cleavage of the benzyl ether group of compound **6a** using H_2/Pd–C provided the precursor 4-fluoro-2-(3-hydroxy-4-methoxyphenyl)benzothiazole (**7**) for radiolabeling. Synthesis of radiolabeling precursors and the reference standards 5- and 6-fluorinated arylbenzothiazoles (**11c–n**) was achieved via the reaction of *o*-aminothiophenol disulfides with substituted benzaldehydes under reducing conditions. The target radiotracers carbon-11 labeled 4-, 5-, and 6-fluorinated arylbenzothiazoles (3-[^{11}C]**6b**, 4-[^{11}C]**11c**, 3-[^{11}C]**11c**, 5-[^{11}C]**11f**, 4-[^{11}C]**11f**, 4-[^{11}C]**11i**, 3-[^{11}C]**11i**, 5-[^{11}C]**11l**, and 4-[^{11}C]**11l**) were prepared by *O*-[^{11}C]methylation of the phenolic hydroxyl precursors (**7**, **11d**, **11e**, **11g**, **11h**, **11j**, **11k**, **11m**, and **11n**) with [^{11}C]methyl triflate and isolated by solid-phase extraction (SPE) purification in 30–55% radiochemical yields.

1. Introduction

Carbon-11 labeled 2-arylbenzothiazoles have been developed by Pittsburgh group as noninvasive biomarkers for biomedical imaging technique positron emission tomography (PET) to image brain amyloid in Alzheimer's disease.[1] Pittsburgh Compound-B (PIB), [^{11}C]6-OH-BTA-1 (Fig. 1), has been investigated in human study, and the results indicate that detecting amyloid plaques in the living human brain with amyloid imaging agents is potentially feasible.[2] Similarly, carbon-11 and fluorine-18 labeled 6-iodo-2-(4′-*N*,*N*-dimethylamino)-phenylimidazo[1,2-*a*]pyridine derivatives[3] and benzofuran derivatives[4] have been also developed as PET brain amyloid imaging agents. Recently, a series of new 2-arylbenzothiazoles have been synthesized by Nottingham group and reported to possess antitumor activity, which show potent and selective inhibitory activity against breast, colon, and lung cancer cell lines.[5] The lead compound 5-fluoro-2-(3,4-dimethoxyphenyl)benzothiazole (**11c**) was found to have exquisitely potent antiproliferative activity with nanomolar GI_{50} values, <0.1 nM for breast cancer cell lines MCF-7 and MDA 468, and 0.25 nM for colon cancer cell line HCC2998. These 2-arylbenzothiazole compounds were screened in the NCI 60 human cancer cell lines, and potent and selective activity was also observed for the compound **11c**. Antitumor benzothiazoles have been proven by Nottingham group to have tyrosine kinase inhibitory properties and could serve as tyrosine kinase inhibitors.[5,6] The enzyme tyrosine kinase is involved in cell signal transduction processes and associated with the proliferation, apoptosis, repair, and angiogenesis of cancer cells.[7] More than two-thirds of human cancers derive from epithelial tissues and the tyrosine kinase is overexpressed in the majority of these cancers.[8] Therefore, numerous selective tyrosine kinase inhibitors with nanomolar to subnanomolar affinities have been actively developed as potential anti-cancer agents.[9] The overexpression of tyrosine kinase in cancers provides an attractive target for the development of enzyme-based PET

Keywords: Fluorinated 2-arylbenzothiazoles; Carbon-11; Positron emission tomography (PET); Tyrosine kinase; Cancer imaging.
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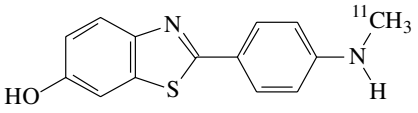
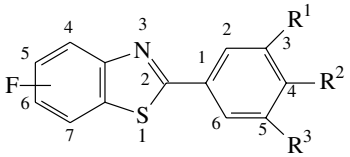


[^{11}C]6-OH-BTA-1
Pittsburgh Compound-B, PIB
(Brain amyloid imaging)



3-[^{11}C]**6b**: 4-F, R^1=O^{11}CH$_3$, R^2=OCH$_3$, R^3=H
4-[^{11}C]**11c**: 5-F, R^1=OCH$_3$, R^2=O^{11}CH$_3$, R^3=H
3-[^{11}C]**11c**: 5-F, R^1=O^{11}CH$_3$, R^2=OCH$_3$, R^3=H
5-[^{11}C]**11f**: 5-F, R^1=OCH$_3$, R^2=OCH$_3$, R^3=O^{11}CH$_3$
4-[^{11}C]**11f**: 5-F, R^1=OCH$_3$, R^2=O^{11}CH$_3$, R^3=OCH$_3$
4-[^{11}C]**11i**: 6-F, R^1=OCH$_3$, R^2=O^{11}CH$_3$, R^3=H
3-[^{11}C]**11i**: 6-F, R^1=O^{11}CH$_3$, R^2=OCH$_3$, R^3=H
5-[^{11}C]**11l**: 6-F, R^1=OCH$_3$, R^2=OCH$_3$, R^3=O^{11}CH$_3$
4-[^{11}C]**11l**: 6-F, R^1=OCH$_3$, R^2=O^{11}CH$_3$, R^3=OCH$_3$

Carbon-11 labeled 4-, 5- and 6-fluorinated 2-arylbenzothiazoles
(Cancer tyrosine kinase imaging)

Figure 1. Chemical structures of carbon-11 labeled 2-arylbenzothiazoles.

cancer imaging agents. To translate chemotherapeutic agents for diagnostic use, we are interested in the design and synthesis of medical probes for PET imaging of cancer. In our previous works, we have developed [^{18}F]SU11248[10] and [^{11}C]Iressa[11] as new PET tracers for imaging cancer tyrosine kinase. However, the in vivo biological evaluation results including biodistribution and animal PET imaging with these tracers were disappointing. The specific binding of the tracers to target enzyme tyrosine kinase in appropriate tumor models awaits further investigations. In this ongoing study, we report the synthesis of carbon-11 labeled fluorinated 2-arylbenzothiazoles as novel potential PET cancer imaging agents as indicated in Figure 1.

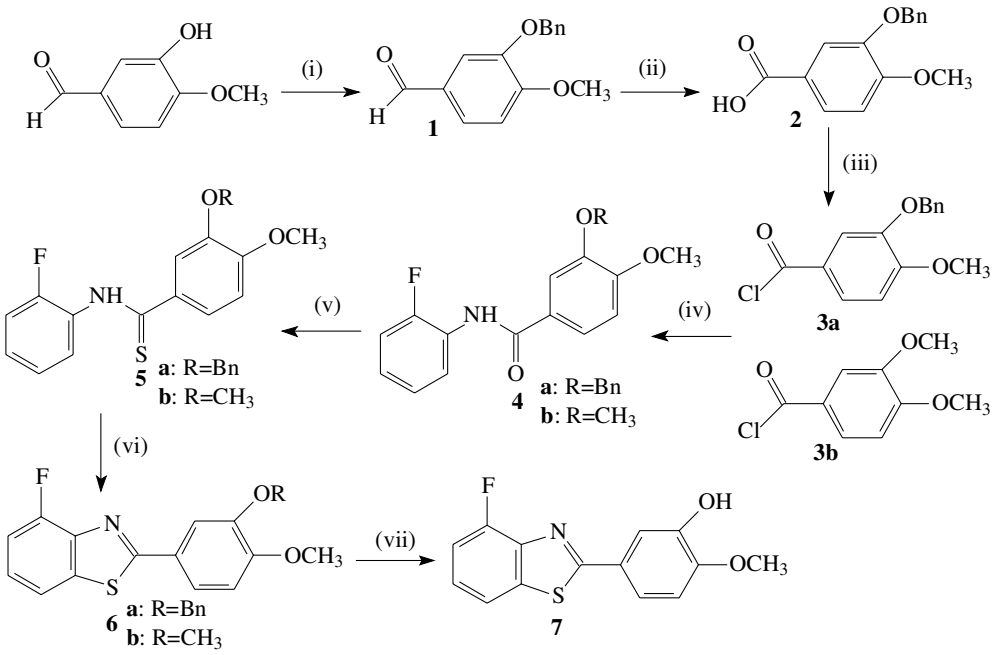
2. Results and discussion

2.1. Chemistry

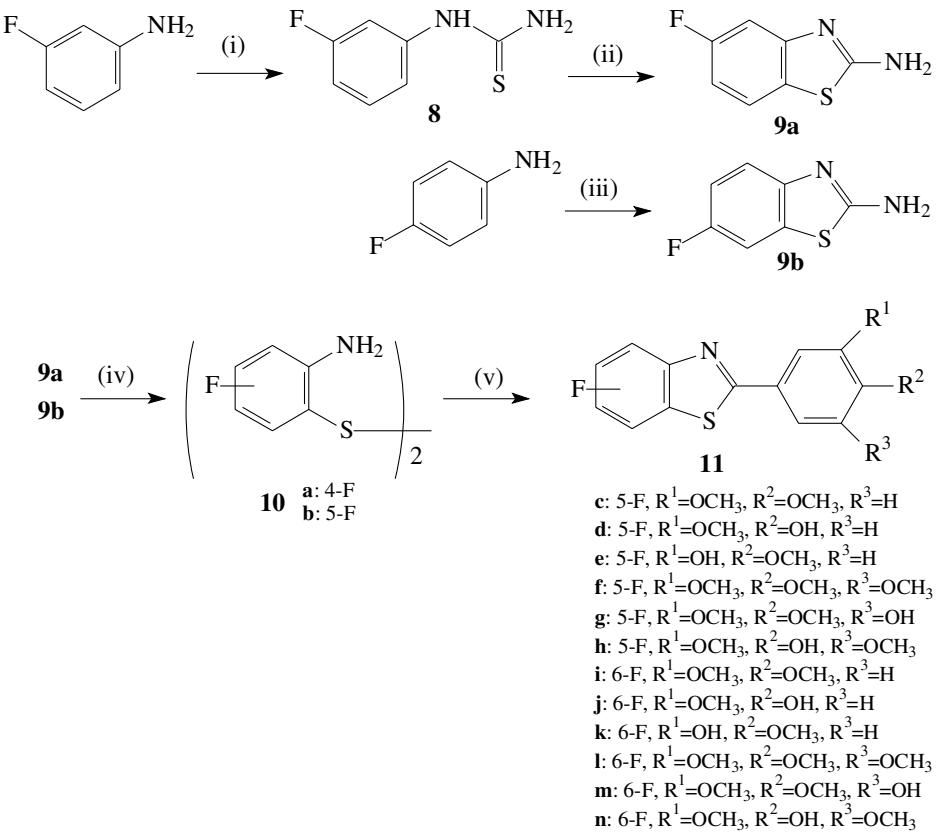
Two approaches were taken to synthesize the precursors and standard compounds 4-, 5-, and 6-fluorinated 2-phenylbenzothiazoles (Schemes 1, and 2).

Synthesis of 4-fluorinated 2-phenylbenzothiazoles is outlined in Scheme 1. Benzylation of starting material 3-hydroxy-4-methoxybenzaldehyde was achieved by the protection of phenolic hydroxyl group with benzyl bromide to provide 3-benzloxy-4-methoxybenzaldehyde (**1**) in 94% yield. Oxidation of compound **1** using sodium chlorite provided 3-benzloxy-4-methoxybenzoic acid (**2**) in 93% yield. Compound **2** was reacted with thionyl chloride to give 3-benzloxy-4-methoxybenzoyl chloride (**3a**) in 94% yield.[12,13] 2-Fluorobenzamides *N*-(2-fluorophenyl)-3-benzloxy-4-methoxybenzamide (**4a**) and *N*-(2-fluorophenyl)-3,4-dimethoxybenzamide (**4b**) were prepared by condensation of aroyl chloride **3a**, or commercially available starting material 3, 4-dimethoxybenzoyl chloride (**3b**), with 2-fluoroaniline in 61% and 83% yield, respectively. The benzamides **4a** and **4b** were converted to their thiobenzamides *N*-(2-fluorophenyl)-3-benzloxy-4-methoxythiobenzamide (**5a**) and *N*-(2-fluorophenyl)-3,4-dimethoxythiobenzamide (**5b**) with Lawesson's reagent in hexamethylphosphoramide (HMPA) in 58% and 76% yield, respectively. Cyclization of thiobenzamides **5a** and **5b** by a modified method of Jacobson thioanilide radical cyclization chemistry using potassium ferricyanide (III) and aqueous sodium hydroxide[14] gave 4-fluorobenzothiazoles 4-fluoro-2-(3-benzloxy-4-methoxyphenyl)benzothiazole (**6a**) and 4-fluoro-2-(3,4-dimethoxyphenyl)benzothiazole (**6b**) in 26% and 57% yield, respectively. Hydrogenolytic cleavage of the benzyl ether group of compound **6a** using H_2/Pd–C provided 4-fluoro-2-(3-hydroxy-4-methoxyphenyl)benzothiazole (**7**) in 73% yield. Compound **7** is the precursor for radiolabeling, and compound **6b** is the reference standard.

5- and 6-Fluorinated 2-arylbenzothiazoles were synthesized using a modification of the literature procedure.[5] The synthetic approach is outlined in Scheme 2. The reaction of benzoyl chloride with ammonium thiocyanate initially gave benzoyl isothiocyanate, which underwent addition with 3-fluoroaniline to afford 3-fluorophenylthiourea (**8**) in 91% yield. 2-amino-5-fluorobenzothiazole (**9a**) was obtained from the cyclization of compound **8** with bromine in 64% yield,[15] and 2-amino-6-fluorobenzothiazole (**9b**) was prepared by one-pot reaction of 4-fluoroaniline with thiocyanogen generated from bromine and potassium thiocyanate in acetic acid in 85% yield.[16] Compounds **9a**,**b** were treated with aqueous potassium hydroxide through hydrolytic cleavage followed by acidification and air oxidation to convert to bis(2-amino-4-fluorophenyl)disulfide (**10a**) and bis(2-amino-5-fluorophenyl)disulfide (**10b**) in 76% and 25% yield, respectively.[17] Interaction of the disulfides **10a** and **10b** with a disubstituted or trisubstituted benzaldehyde and triphenylphosphine using *p*-toluenesulfonic



Scheme 1. Synthesis of 4-fluorinated 2-arylbenzothiazoles. Reagents and conditions (yields): (i) benzyl bromide, K_2CO_3, DMF, 65 °C, (94%); (ii) $NaClO_2$, H_2NSO_3H, AcOH, 18–20 °C, (93%); (iii) $SOCl_2$, toluene, 100 °C, (94%); (iv) 2-fluoroaniline, Et_3N, CH_2Cl_2, (61% and 83%); (v) Lawesson's reagent, HMPA, 100 °C, (58% and 76%); (vi) $K_3Fe(CN)_6$, NaOH, 90 °C, (26% and 57%); (vii) H_2, 10% Pd–C, MeOH, (73%).

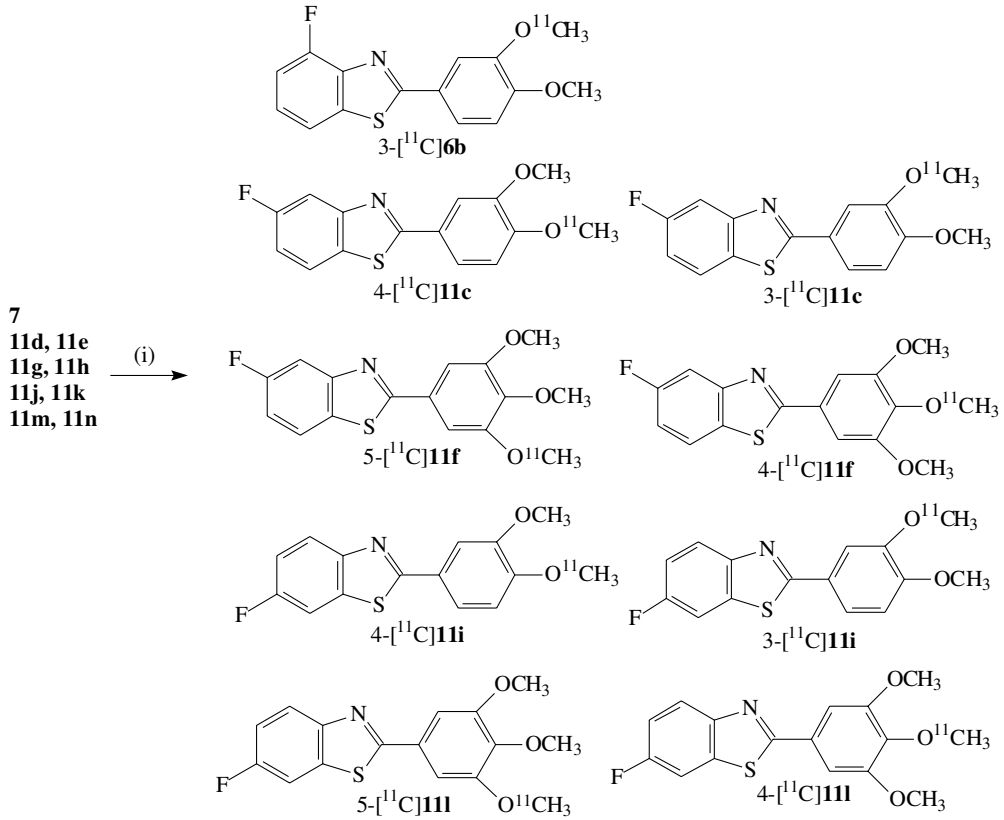


Scheme 2. Synthesis of 5- and 6-fluorinated 2-arylbenzothiazoles. Reagents and conditions (yields): (i) NH_4SCN, benzoyl chloride, acetone, reflux, (91%); (ii) Br_2, CH_2Cl_2, < 32 °C, (64%); (iii) KSCN, Br_2, AcOH, (85%); (iv) KOH, reflux, (76% and 25%); (v) disubstituted or trisubstituted benzaldehyde, PPh_3, *p*-TsOH, toluene, reflux, (63–92%).

acid as catalyst gave corresponding 5- or 6-fluorinated 2-phenylbenzothiazoles 5-fluoro-2-(3,4-dimethoxyphenyl)benzothiazole (**11c**), 5-fluoro-2-(4-hydroxy-3-methoxyphenyl)benzothiazole (**11d**), 5-fluoro-2-(3-hydroxy-4-methoxyphenyl)benzothiazole (**11e**), 5-fluoro-2-(3,4,5-trimethoxyphenyl)benzothiazole (**11f**), 5-fluoro-2-(5-hydroxy-3,4-dimethoxyphenyl)benzothiazole (**11g**), 5-fluoro-2-(4-hydroxy-3,5-dimethoxyphenyl)benzothiazole (**11h**), 6-fluoro-2-(3,4-dimethoxyphenyl)benzothiazole (**11i**), 6-fluoro-2-(4-hydroxy-3-methoxyphenyl)benzothiazole (**11j**), 6-fluoro-2-(3-hydroxy-4-methoxyphenyl)benzothiazole (**11k**), 6-fluoro-2-(3,4,5-trimethoxyphenyl)benzothiazole (**11l**), 6-fluoro-2-(5-hydroxy-3,4-dimethoxyphenyl)benzothiazole (**11m**), and 6-fluoro-2-(4-hydroxy-3,5-dimethoxyphenyl)benzothiazole (**11n**) in 63–92% yields. Compounds **11d**, **11e**, **11g**, **11h**, **11j**, **11k**, **11m**, and **11n** are precursors for radiolabeling, and compounds **11c**, **11f**, **11i**, and **11l** are reference standards.

The target compounds **11c**, **11f**, and **11i** are the known compounds,[5] and the target compound **11l** is a novel compound. The in vitro assay shows compound **11c** has exquisitely potent antiproliferative activity, $GI_{50} < 0.1$ nM for MCF-7 and MDA 468 breast cancer cell lines, and potent and selective activity of compound **11c** was also observed in the NCI 60 human cancer cell line panel.[5] The reported in vitro data indicate compounds **11f** and **11i** have similar antitumor activity, **11f** GI_{50} 1.9 nM for MCF-7 and 2.1 nM for MDA 468, **11i** GI_{50} 62 nM for MCF-7 and 5 nM for MDA 468, in comparison with the most potent compound **11c**, although their GI_{50} data are relatively low.[5] The chemical structures of 5-fluorinated 2-arylbenzothiazoles show compound **11f** has one more methoxy group bearing at 5-position of the aryl moiety in comparison with compound **11c**. Likewise, the chemical structures of 6-fluorinated 2-arylbenzothiazoles show compound **11l** has one more methoxy group bearing at 5-position of the aryl moiety in comparison with compound **11i**. Based on the structure and activity relationship (SAR) analysis, we can predict compound **11l** would likely have similar antiproliferative activity with compound **11i**.

The phenolic hydroxyl precursors **7**, **11d**, **11e**, **11g**, **11h**, **11j**, **11k**, **11m**, and **11n** can be directly labeled with a positron emitting radionuclide carbon-11 at aryl moiety of 2-arylbenzothiazoles, and 4-, 5-, and 6-fluorinated 2-phenylbenzothiazoles bearing a fluoro-group at 4-, 5-, and 6-positions are also the target molecules for radiolabeling of 2-arylbenzothiazoles with another positron emitting radionuclide fluorine-18 at benzothiazole moiety, their corresponding precursors would be 4-, 5-, and 6-nitro-2-phenylbenzothiazoles bearing a nitro-group at 4-, 5-, and 6-positions of benzothiazole moiety, and fluorine-18 radiolabeling methodology will be exactly same with the synthesis of the tracer [^{18}F]SU11248 aforementioned.[10]



Scheme 3. Synthesis of carbon-11 labeled 4-, 5-, and 6-fluorinated 2-arylbenzothiazoles. Reagents (yields): (i) $^{11}CH_3OTf$, CH_3CN, 3 N NaOH, (30–55%).

2.2. Radiochemistry

Synthesis of the target tracers 4-fluoro-2-(3-[^{11}C]methoxy-4-methoxyphenyl)benzothiazole (3-[^{11}C]**6b**), 5-fluoro-2-(3-methoxy-4-[^{11}C]methoxyphenyl)benzothiazole (4-[^{11}C]**11c**), 5-fluoro-2-(3-[^{11}C]methoxy-4-methoxyphenyl)benzothiazole (3-[^{11}C]**11c**), 5-fluoro-2-(5-[^{11}C]methoxy-3,4-dimethoxyphenyl)benzothiazole (5-[^{11}C]**11f**), 5-fluoro-2-(4-[^{11}C]methoxy-3,5-dimethoxyphenyl)benzothiazole (4-[^{11}C]**11f**), 6-fluoro-2-(3-methoxy-4-[^{11}C]methoxyphenyl)benzothiazole (4-[^{11}C]**11i**), 6-fluoro-2-(3-[^{11}C]methoxy-4-methoxyphenyl)benzothiazole (3-[^{11}C]**11i**), 6-fluoro-2-(5-[^{11}C]methoxy-3,4-dimethoxyphenyl)benzothiazole (5-[^{11}C]**11l**), and 6-fluoro-2-(4-[^{11}C]methoxy-3,5-dimethoxyphenyl)benzothiazole (4-[^{11}C]**11l**) is outlined in Scheme 3. The phenolic hydroxyl precursor **7**, **11d**, **11e**, **11g**, **11h**, **11j**, **11k**, **11m** or **11n** was labeled by [^{11}C]methyl triflate ($^{11}CH_3OTf$)[18,19] through *O*-[^{11}C]methylation[20] of hydroxyphenyl position under basic conditions using 3 N NaOH. The tracer was isolated by solid-phase extraction (SPE) purification[21,22] to produce pure target radiolabeled compound with 30–55% radiochemical yields, based on $^{11}CO_2$, decay corrected to end of bombardment (EOB). The large polarity difference between the phenolic hydroxyl precursor and the labeled methylated product permitted the use of SPE technique for purification of labeled product from radiolabeling reaction mixture. The reaction mixture was diluted with $NaHCO_3$ and loaded onto C-18 Sep-Pak cartridge by gas pressure. The cartridge column was washed with water to remove non-reacted $^{11}CH_3OTf$ and precursor and reaction solvent, and then final labeled product was eluted with ethanol. Chemical purity, radiochemical purity, and specific radioactivity were determined by analytical HPLC method.[23] The chemical purity of precursors and reference standards was >90%. The radiochemical purity of target tracers was >95% determined by radio-HPLC through γ-ray (NaI) flow detector, and the chemical purity of target tracers was > 93% determined by reversed-phase HPLC through UV flow detector. The average specific radioactivity of target tracers was 1–2 Ci/μmol at end-of-synthesis (EOS).

3. Conclusion

An efficient and convenient chemical and radiochemical synthesis of 4-, 5-, and 6-fluorinated arylbenzothiazole phenolic hydroxyl precursors, 4-, 5-, and 6-fluorinated arylbenzothiazole reference standards, and carbon-11 labeled 4-, 5-, and 6-fluorinated arylbenzothiazole target tracers has been developed. The synthetic methodology employed classical heterocycle chemistry such as thioanilide radical cyclization chemistry. Carbon-11 labeling was incorporated efficiently using $^{11}CH_3OTf$, a signature reaction of carbon-11 radiochemistry from our laboratory. Radiosynthesis produced new probes carbon-11 labeled 4-, 5-, and 6-fluorinated arylbenzothiazoles in amounts and purity suitable for the preclinical application in animal studies by PET imaging techniques. Labeled product suitable for injection, with the specific radioactivities in a range of 1–2 Ci/μmol at EOS, can be obtained in ~20 min from EOB including SPE purification and formulation. These chemistry results combined with the reported in vitro biological data encourage further in vivo biological evaluation of carbon-11 labeled 4-, 5-, and 6-fluorinated arylbenzothiazoles as novel potential PET cancer imaging agents for tyrosine kinase.

4. Experimental

4.1. General

All commercial reagents and solvents were used without further purification unless otherwise specified. Melting points were determined on a MEL-TEMP II capillary tube apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker QE 300 FT-NMR spectrometer using tetramethylsilane (TMS) as an internal standard. Chemical shift data for the proton resonances were reported in parts per million (ppm, δ scale) relative to internal standard TMS (δ 0.0), and coupling constants (J) are reported in hertz (Hz). The low resolution mass spectra (LRMS) were obtained using a Bruker Biflex III MALDI-Tof mass spectrometer, and the high resolution mass spectra (HRMS) were obtained using a Kratos MS80 mass spectrometer. Chromatographic solvent proportions are expressed on a volume: volume basis. Thin layer chromatography was run using Analtech silica gel GF uniplates (5 × 10 cm^2). Plates were visualized by UV light. Normal phase flash chromatography was carried out on EM Science silica gel 60 (230–400 mesh) with a forced flow of the indicated solvent system in the proportions described below. All moisture- and/or air-sensitive reactions were performed under a positive pressure of nitrogen maintained by a direct line from a nitrogen source.

Analytical HPLC was performed using a Prodigy (Phenomenex) 5 μm C-18 column, 4.6 × 250 mm; 3:1:3 CH_3CN/MeOH/20 mM, pH 6.7, $KHPO_4^-$ mobile phase, 1.5 mL/min flow rate, UV (254 nm) and γ-ray (NaI) flow detectors. Semi-prep C-18 guard cartridge column 1 × 1 cm was obtained from E.S. Industries, Berlin, NJ, and part number 300121-C18-BD 10 μ. Sterile vented Millex-GS 0.22 μm filter unit was obtained from Millipore Corporation, Bedford, MA.

4.2. 3-Benzloxy-4-methoxybenzaldehyde (1)

A suspension of 3-hydroxy-4-methoxybenzaldehyde (10.0 g, 65.7 mmol), anhydrous K_2CO_3 (13.6 g, 98.5 mmol), and KI (0.3 g) in anhydrous DMF (60 mL) was stirred and heated to 65 °C. Benzyl bromide (9.4 mL, 85.9 mmol) was added dropwise and the stirred mixture was heated at 65 °C for 22 h. After cooling to room temperature, water (150 mL) was added and the mixture was extracted with EtOAc (2× 100 mL). The combined organic phase was washed with 1 N NaOH (2× 50 mL) and water (3× 100 mL), dried over anhydrous Na_2SO_4, and evaporated at reduced pressure. A yellowish solid was obtained.

The crude product was purified by column chromatography (EtOAc/hexanes, 1:4) to give **1** as a pale yellow solid (14.9 g, 94%), mp 52–52 °C. ^{1}H NMR ($CDCl_3$) δ: 9.82 (s, 1H, CHO), 7.48–7.26 (m, 7H, Ar-H), 7.00 (d, J = 8.1 Hz, 1H, Ar-H), 5.20 (s, 2H, OCH_2), 3.97 (s, 3H, OCH_3).

4.3. 3-Benzloxy-4-methoxybenzoic acid (2)

A suspension of compound **1** (11.6 g, 48.0 mmol) and sulfamic acid (6.4 g, 64.6 mmol) in 80% AcOH (90 mL) was stirred and a solution of 80% $NaClO_2$ (5.6 g, 50.0 mmol) in water (23 mL) was added in dropwise. The temperature was maintained at 18–20 °C using an ice-water bath. The yellow slurry was stirred at 20 °C for 1 h and the reaction mixture was diluted by the addition of water (80 mL). The mixture was filtered, and the solid was washed with water and dried to give **2** as a white solid (11.5 g, 93%), mp 144–145 °C. ^{1}H NMR ($CDCl_3$) δ: 7.78 (dd, J = 2.2, 8.8 Hz, 1H, Ar-H), 7.66 (d, J = 1.5 Hz, 1H, Ar-H), 7.49–7.32 (m, 5H, m, Ar-H), 6.94 (d, J = 8.8 Hz, 1H, Ar-H), 5.20 (s, 2H, OCH_2), 3.95 (s, 3H, OCH_3).

4.4. 3-Benzloxy-4-methoxybenzoyl chloride (3a)

A solution of compound **2** (9 g, 34.9 mmol) in toluene (120 mL) was heated to 90 °C. $SOCl_2$ (3.5 mL, 48.1 mmol) was added dropwise and the solution was heated at 100 °C for 6 h. After cooling, the solution was concentrated at reduced pressure to give **3a** as a light brown oil which became a crystal when cooled to room temperature (9.1 g, 94%), mp 57–58 °C. ^{1}H NMR ($CDCl_3$) δ: 7.83 (dd, J = 2.2, 8.8 Hz, 1H, Ar-H), 7.61 (d, J = 2.2 Hz, 1H, Ar-H), 7.48–7.32 (m, 5H, Ar-H), 6.94 (d, J = 8.8 Hz, 1H, Ar-H), 5.18 (s, 2H, OCH_2), 3.98 (s, 3H, OCH_3).

4.5. *N*-(2-Fluorophenyl)-3-benzloxy-4-methoxybenzamide (4a)

Compound **3a** (6.0 g, 21.7 mmol) was added to a solution of 2-fluoroaniline (2.5 mL, 23.9 mmol) and Et_3N (3.0 mL) in CH_2Cl_2 (100 mL). The reaction mixture was heated at reflux for 3 h. After cooling, the solution was washed with water (2× 100 mL), dried over Na_2SO_4, and evaporated at reduced pressure. The crude product was purified by column chromatography (MeOH/CH_2Cl_2, 1:99) to give **4a** as a white solid (5.1 g, 61%), mp 170–171 °C. ^{1}H NMR ($CDCl_3$) δ: 7.94 (d, J = 2.9 Hz, 1H, Ar-H), 7.53–7.32 (m, 7H, Ar-H), 7.21–7.05 (m, 4H, Ar-H), 6.96 (d, J = 8.8 Hz, 1H, Ar-H), 5.23 (s, 2H, OCH_2), 3.96 (s, 3H, OCH_3).

4.6. *N*-(2-Fluorophenyl)-3, 4-dimethoxybenzamide (4b)

Using similar method for the preparation of compound **4a**, compound **4b** was obtained by the reaction of 2-fluoroaniline and 3,4-dimethoxybenzoyl chloride **3b** in CH_2Cl_2 as a white solid in 83% yield, mp 130–131 °C. ^{1}H NMR ($CDCl_3$) δ: 8.02 (d, J = 2.2 Hz, 1H, Ar-H), 7.51 (s, 1H, Ar-H), 7.42 (dd, J = 2.4, 9.5 Hz, 1H, Ar-H), 7.21–7.07 (m, 3H, Ar-H), 6.94 (d, J = 8.1 Hz, 1H, Ar-H), 3.97 (s, 3H, OCH_3), 3.96 (s, 3H, OCH_3).

4.7. *N*-(2-Fluorophenyl)-3-benzloxy-4-methoxythiobenzamide (5a)

A mixture of compound **4a** (3.0 g, 7.74 mmol) and Lawesson's reagent (2.2 g, 5.4 mmol) in HMPA (6 mL) was stirred at 100 °C for 16 h. After cooling, it was poured into water. The product was extracted into diethyl ether (3× 100 mL), and the ethereal layer was washed with water (3× 100 mL), dried over Na_2SO_4 and evaporated at reduced pressure. The crude product was purified by column chromatography (EtOAc/hexanes, 1:4) to give **5a** as a yellow solid (1.8 g, 58%), mp 153–154 °C. ^{1}H NMR ($CDCl_3$) δ: 8.94 (br s, 1H, CSNH), 7.61 (s, 1H, Ar-H), 7.49–7.32 (m, 7H, Ar-H), 7.25–7.16 (m, 3H, Ar-H), 6.90 (d, J = 8.8 Hz, 1H, Ar-H), 5.21 (s, 2H, OCH_2), 3.94 (s, 3H, OCH_3).

4.8. *N*-(2-Fluorophenyl)-3,4-dimethoxythiobenzamide (5b)

Similarly, compound **5b** was prepared by the reaction of compound **4b** and Lawesson's reagent in HMPA as a yellow solid in 76% yield, mp 106–107 °C. ^{1}H NMR ($CDCl_3$) δ: 9.06 (br s, 1H, CSNH), 7.58 (s, 1H, Ar-H), 7.39 (dd, J = 2.2, 8.8 Hz, 1H, Ar-H), 7.25–7.15 (m, 4H, Ar-H), 6.86 (d, J = 8.1 Hz, 1H, Ar-H), 3.95 (s, 3H, OCH_3), 3.93 (s, 3H, OCH_3).

4.9. 4-Fluoro-2-(3-benzloxy-4-methoxyphenyl)benzothiazole (6a)

A little EtOH, NaOH (1.5 g, 37.5 mmol), and water (5 mL) were added to compound **5a** (1.0 g, 2.7 mmol). The mixture was added dropwise to a stirred solution of potassium ferricyanide (III) (3.6 g, 10.9 mmol) in water (5 mL). The resulting solution was heated at 90 °C for 2 h and then cooled with ice bath. The precipitate was collected by filtration, washed with water (3× 10 mL), then dissolved in EtOAc (20 mL), and insoluble solid was removed by filtration. The filtrate was evaporated at reduced pressure and the crude product was purified by column chromatography (EtOAc/hexanes, 1:4) to give **6a** as a pale yellow powder (260 mg, 26%), mp 138–139 °C. ^{1}H NMR ($CDCl_3$) δ: 7.78–7.76 (m, 1H, Ar-H), 7.68–7.62 (m, 2H, Ar-H), 7.52 (d, J = 7.3 Hz, 2H, Ar-H), 7.43–7.27 (m, 4H, Ar-H), 7.20–7.15 (m, 1H, Ar-H), 6.97 (d, J = 8.1 Hz, 1H, Ar-H), 5.27 (s, 2H, OCH_2), 3.96 (s, 3H, OCH_3).

4.10. 4-Fluoro-2-(3,4-dimethoxyphenyl)benzothiazole (6b)

Similarly, compound **6b** was prepared by the reaction of compound **5b** and potassium ferricyanide (III) as a pale yellow powder in 57% yield, mp 127–128 °C (lit.[5] mp 129 °C). ^{1}H NMR ($CDCl_3$) δ: 7.74 (d, J = 2.4 Hz, 1H, H-7), 7.66–7.60 (m, 2H, H-2′, H-6′), 7.31 (dt, J = 4.5, 8.1 Hz, 1H, H-6), 7.18 (dd, J = 2.2, 10.3 Hz, 1H, H-5), 6.95 (d, J = 8.8 Hz, 1H, H-5′), 4.03 (s, 3H, OCH_3), 3.97 (s, 3H, OCH_3).

4.11. 4-Fluoro-2-(3-hydroxy-4-methoxyphenyl)benzothiazole (7)

A solution of compound **6a** (200 mg, 0.5 mmol) in MeOH (15 mL) was hydrogenated using 10% Pd–C (50 mg) as catalyst at atmospheric pressure overnight. The catalyst was removed by filtration, and the solution was evaporated at reduced pressure. The crude product was purified by column chromatography (EtOAc/hexanes, 1:4) to give **7** as a pale yellow powder (110 mg, 73%), mp 135–136 °C. ^{1}H NMR ($CDCl_3$) δ: 8.05–7.87 (m, 1 H, Ar-H), 7.70–7.62 (m, 2H, Ar-H), 7.49–7.16 (m, 2H, Ar-H), 6.94 (d, J = 8.1 Hz, 1H, Ar-H), 5.76 (s, 1H, OH), 3.97 (s, 3H, OCH_3). LRMS (CI): 276.0 ($[M+H]^+$, 100%). HRMS (CI): calcd for $C_{14}H_{11}O_2NFS$ 276.0489 $[M+H]^+$, found 276.0490.

4.12. 3-Fluorophenylthiourea (8)

Benzoyl chloride (17.2 mL, 148.4 mmol) was added dropwise to a solution of ammonium thiocyanate (11.3 g, 148.4 mmol) in acetone (20 mL). The suspension was heated at reflux and 3-fluoroaniline (13.0 mL, 135.0 mmol) was added. The reaction mixture was diluted with acetone (15 mL) and continued to reflux for 1 h. To this mixture a solution of NaOH (16.9 g, 422.5 mmol) in water (100 mL) was added and the yellow homogeneous solution was heated at reflux for 1.5 h. Then the reaction mixture was cooled and concentrated at reduced pressure to remove the acetone. The mixture was adjusted pH to 5.0 with concentrated HCl, and then pH to 11.0 with NH_4OH to give a pale yellow precipitate, which was collected by filtration, washed with water (3× 30 mL), and dried under vacuum to yield **8** as a pale yellow solid (19.2 g, 91%), mp 113–114 °C (lit.[15] mp 114–115 °C). ^{1}H NMR (DMSO-d_6) δ: 9.83 (s, 1H, NH), 7.95–7.50 (m, 3H, NH_2 and H-2), 7.33 (dt, J = 8.1, 15.5 Hz, 1H, H-5), 7.14 (d, J = 7.3 Hz, 1H, H-6), 6.91 (dt, J = 2.9, 8.0 Hz, 1H, H-4).

4.13. 2-Amino-5-fluorobenzothiazole (9a)

A solution of bromine (4.6 mL, 89.7 mmol) in CH_2Cl_2 (40 mL) was added dropwise to a solution of compound **8** (14.0 g, 89.7 mmol) in CH_2Cl_2 (250 mL), maintaining the temperature below 30 °C. The resulting mixture was heated at reflux for 3 h and then cooled to room temperature. The precipitate was collected, suspended in water (400 mL), basified to pH 11.0 with NH_4OH, and extracted with EtOAc (3× 100 mL). The combined organic phase was washed with water (100 mL), brine (100 mL), dried over Na_2SO_4, filtered, and evaporated at reduced pressure. A crude yellow product was obtained, which was recrystallized from benzene to yield **9a** as a white solid (8.9 g, 64%), mp 182–183 °C (lit.[15] mp 181 °C). ^{1}H NMR (DMSO-d_6) δ: 7.65–7.60 (m, 3H, NH_2 and H-7), 7.11 (dd, J = 2.2, 10.3 Hz, 1H, H-4), 6.83 (dt, J = 3.0, 9.6 Hz, 1H, H-6).

4.14. 2-Amino-6-fluorobenzothiazole (9b)

A solution of 4-fluoroaniline (9.5 mL, 100.0 mmol) and potassium thiocyanate (15.2 g, 200.0 mmol) in AcOH (150 mL) was cooled in an ice bath and stirred, and then a solution of bromine (5.1 mL, 100.0 mmol) in AcOH (15 mL) was added dropwise. The reaction mixture was stirred at room temperature for 2 h, then poured into cold water (200 mL), basified to pH 11.0 with NH_4OH, and extracted with EtOAc (3× 100 mL). The combined organic phase was washed with water (100 mL), brine (100 mL), dried over Na_2SO_4, filtered, and evaporated at reduced pressure. A crude yellow product was obtained, which was purified by column chromatography (EtOAc/hexanes, 7:3) to give **9b** as white powder (12.1 g, 85%), mp 130–131 °C (lit.[16] mp 127–128 °C). ^{1}H NMR (DMSO-d_6) δ: 7.57 (dd, J = 2.9, 8.8 Hz, 1H, H-7), 7.45 (s, 2H, NH_2), 7.29 (dd, J = 5.1, 8.8 Hz, 1H, H-4), 7.02 (dt, J = 2.2, 8.8 Hz, 1H, H-5).

4.15. Bis(2-amino-4-fluorophenyl)disulfide (10a)

Compound **9a** (5.0 g, 29.8 mmol) was added to a solution of KOH (25.0 g, 446.4 mmol) in water (50 mL). The reaction mixture was heated at reflux for 5 h and then cooled to room temperature, which was acidified to pH 6.0 with AcOH. Water (50 mL) was added, and the resulting mixture was stirred overnight. The precipitate was collected, and the crude product was purified by column chromatography ($MeOH/CH_2Cl_2$, 0.5:99.5) to give **10a** as a yellow solid (3.2 g, 76%), mp 74–75 °C (lit.[17] mp 75–76 °C). ^{1}H NMR ($CDCl_3$) δ: 7.04 (dd, J = 6.6, 8.1 Hz, 1H, H-6), 6.41 (dd, J = 2.2, 10.3 Hz, 1H, H-3), 6.29 (dt, J = 2.2, 8.1 Hz, 1H, H-5), 4.48 (s, 2H, NH_2).

4.16. Bis(2-amino-5-fluorophenyl)disulfide (10b)

Using similar method for the preparation of compound **10a**, compound **10b** was obtained by the reaction of compound **9b** and KOH as a yellow solid in 25% yield, mp 75–76 °C. ^{1}H NMR (DMSO-d_6) δ: 7.04 (dt, J = 2.9, 8.8 Hz, 1H, H-4), 6.83 (dd, J = 2.9, 8.8 Hz, 1H, H-6), 6.73 (dd, J = 5.2, 8.9 Hz, 1H, H-3), 5.37 (s, 2H, NH_2).

4.17. General method for the preparation of 5- and 6-fluorinated 2-arylbenzothiazoles (11c–n)

Disubstituted or trisubstituted benzaldehyde (2.2 mmol), *p*-toluenesulfonic acid (19.0 mg, 0.1 mmol), and triphenylphosphine (262 mg, 1.0 mmol) were added to a solution of compound **10a** or **10b** (284 mg, 1.0 mmol) in toluene (10 mL). The reaction mixture was heated at reflux for 15 h, and then cooled and evaporated at reduced pressure. The crude product was purified column chromatography ($MeOH/CH_2Cl_2$, 1:49) to give substituted 2-arylbenzothiazole in 63–92% yields.

4.17.1. 5-Fluoro-2-(3,4-dimethoxyphenyl)benzothiazole (11c). The product was obtained from **10a** and 3,4-dimethoxybenzaldehyde as a yellow solid in 90% yield, mp 108–109 °C (lit.[5] mp 110 °C). ^{1}H NMR (DMSO-d_6) δ: 8.13 (dd, J = 5.2, 8.8 Hz, 1H, H-7), 7.85 (dd, J = 2.2, 9.5 Hz, 1H, H-4), 7.61–7.59 (m, 2H, H-2′, H-6′), 7.32 (dt, J = 2.3, 8.9 Hz, 1H, H-6), 7.11

(d, $J = 8.9$ Hz, 1H, H-5′), 3.87 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3).

4.17.2. 5-Fluoro-2-(4-hydroxy-3-methoxyphenyl)benzothiazole (11d). The product was obtained from **10a** and 4-hydroxy-3-methoxybenzaldehyde as a yellow solid in 85% yield, mp 157–158 °C (lit.[5] mp 156 °C). ^{1}H NMR (DMSO-d_6) δ: 9.91 (s, 1H, OH), 8.11 (dd, $J = 5.9$, 8.8 Hz, 1H, H-7), 7.83 (dd, $J = 2.5$, 9.6 Hz, 1H, H-4), 7.60 (s, 1H, H-2′), 7.50 (d, $J = 8.1$ Hz, 1H, H-6′), 7.30 (t, $J = 8.8$ Hz, 1H, H-6), 6.93 (d, $J = 8.1$ Hz, 1H, H-5′), 3.88 (s, 3H, OCH_3).

4.17.3. 5-Fluoro-2-(3-hydroxy-4-methoxyphenyl)benzothiazole (11e). The product was obtained from **10a** and 3-hydroxy-4-methoxybenzaldehyde as a yellow solid in 88% yield, mp 170–171 °C (lit.[5] mp 169–172 °C). ^{1}H NMR (DMSO-d_6) δ: 9.57 (s, 1H, OH), 8.11 (dd, $J = 5.9$, 8.8 Hz, 1H, H-7), 7.83 (dd, $J = 2.9$, 10.3 Hz, 1H, H-4), 7.52–7.48 (m, 2H, H-2′, H-6′), 7.31 (dt, $J = 2.9$, 9.5 Hz, 1H, H-6), 7.07 (d, $J = 8.1$ Hz, 1H, H-5′), 3.84 (s, 3H, OCH_3).

4.17.4. 5-Fluoro-2-(3,4,5-trimethoxyphenyl)benzothiazole (11f). The product was obtained from **10a** and 3,4,5-trimethoxybenzaldehyde as a yellow solid in 73% yield, mp 117–118 °C (lit.[5] mp 120–122 °C). ^{1}H NMR ($CDCl_3$) δ: 7.81 (dd, $J = 5.2$, 8.8 Hz, 1H, H-7), 7.73 (dd, $J = 2.9$, 9.5 Hz, 1H, H-4), 7.31 (s, 2H, H-2′, H-6′), 7.16 (dt, $J = 2.3$, 8.9 Hz, 1H, H-6), 3.99 (s, 6H, OCH_3), 3.93 (s, 3H, OCH_3).

4.17.5. 5-Fluoro-2-(5-hydroxy-3,4-dimethoxyphenyl)benzothiazole (11g). The product was obtained from **10a** and 5-hydroxy-3,4-dimethoxybenzaldehyde as a yellow solid in 63% yield, mp 180–181 °C (lit.[4] mp 178–180 °C). ^{1}H NMR ($CDCl_3$) δ: 7.81 (dd, $J = 5.1$, 8.8 Hz, 1H, H-7), 7.72 (dd, $J = 2.2$, 9.6 Hz, 1H, H-4), 7.33 (d, $J = 2.2$ Hz, 1H, H-2′), 7.26 (d, $J = 2.9$ Hz, 1H, H-6′), 7.15 (dt, $J = 3.0$, 8.8 Hz, 1H, H-6), 5.95 (s, 1H, OH), 3.40 (s, 3H, OCH_3), 3.98 (s, 3H, OCH_3).

4.17.6. 5-Fluoro-2-(4-hydroxy-3,5-dimethoxyphenyl)benzothiazole (11h). The product was obtained from **10a** and 4-hydroxy-3,5-dimethoxybenzaldehyde as a yellow solid in 71% yield, mp 174–175 °C. ^{1}H NMR ($CDCl_3$) δ: 7.96 (dd, $J = 5.2$, 8.8 Hz, 1H, H-7), 7.56 (dd, $J = 2.9$, 8.1 Hz, 1H, H-4), 7.30 (s, 2H, H-2′, H-6′), 7.21 (dt, $J = 3.0$, 8.9 Hz, 1H, H-6), 5.89 (s, 1H, OH), 4.01 (s, 6H, OCH_3). LRMS (CI): 306.0 ($[M+H]^+$, 91%), 305.0 (M^+, 100%). HRMS (CI): calcd for $C_{15}H_{13}O_3NFS$ 306.0595 $[M+H]^+$, found 306.0596.

4.17.7. 6-Fluoro-2-(3,4-dimethoxyphenyl)benzothiazole (11i). The product was obtained from **10b** and 3,4-dimethoxybenzaldehyde as a yellow solid in 89% yield, mp 154–155 °C (lit.[5] mp 153–155 °C). ^{1}H NMR ($CDCl_3$) δ: 7.97 (dd, $J = 4.4$, 8.8 Hz, 1H, H-4), 7.68 (d, $J = 1.5$ Hz,1H, H-2′), 7.58–7.54 (m, 2H, H-6′, H-7), 7.21 (dt, $J = 2.9$, 8.8 Hz, 1H, H-5), 6.95 (d, $J = 8.8$ Hz, 1H, H-5′), 4.02 (s, 3H, OCH_3), 3.96 (s, 3H, OCH_3).

4.17.8. 6-Fluoro-2-(4-hydroxy-3-methoxyphenyl)benzothiazole (11j). The product was obtained from **10b** and 4-hydroxy-3-methoxybenzaldehyde as a yellow solid in 86% yield, mp 199–200 °C. ^{1}H NMR (DMSO-d_6) δ: 9.86 (s, 1H, OH), 8.02–7.97 (m, 2H, H-2′, H-4), 7.59 (d, $J = 2.2$ Hz, 1H, H-6′), 7.47 (dd, $J = 2.2$, 8.9 Hz, 1H, H-7), 7.36 (dt, $J = 2.9$, 9.5Hz, 1H, H-5), 6.93 (d, $J = 8.1$ Hz, 1H, H-5′), 3.88 (s, 3H, OCH_3). LRMS (CI): 276.0 ($[M+H]^+$, 78%), 275.0 (M^+, 100%). HRMS (CI): calcd for $C_{14}H_{11}O_2NFS$ 276.0489 $[M+H]^+$, found 276.0477.

4.17.9. 6-Fluoro-2-(3-hydroxy-4-methoxyphenyl)benzothiazole (11k). The product was obtained from **10b** and 3-hydroxy-4-methoxybenzaldehyde as a yellow solid in 80% yield, mp 184–185 °C. ^{1}H NMR ($CDCl_3$) δ: 7.96 (dd, $J = 4.4$, 8.8 Hz, 1H, H-4), 7.63–7.53 (m, 3H, H-2′, H-6′ and H-7), 7.20 (dt, $J = 2.9$, 9.5 Hz, 1H, H-5), 6.94 (d, $J = 8.1$ Hz, 1H, H-5′), 5.76 (br s, 1H, OH), 3.97 (s, 3H, OCH_3). LRMS (CI): 276.0 ($[M+H]^+$, 83%), 275.0 (M^+, 100%). HRMS (CI): calcd for $C_{14}H_{11}O_2NFS$ 276.0489 $[M+H]^+$, found 276.0490.

4.17.10. 6-Fluoro-2-(3,4,5-trimethoxyphenyl)benzothiazole (11l). The product was obtained from **10b** and 3,4,5-trimethoxybenzaldehyde as a yellow solid in 92% yield, mp 151–152 °C. ^{1}H NMR ($CDCl_3$) δ: 7.99 (dd, $J = 5.1$, 8.8 Hz, 1H, H-4), 7.57 (dd, $J = 2.3$, 8.1 Hz, 1H, H-7), 7.29 (s, 2H, H-2′, H-6′), 7.22 (dt, $J = 2.2$, 8.8 Hz, 1H, H-5), 3.99 (s, 6H, OCH_3), 3.93 (s, 3H, OCH_3). LRMS (CI): 320.0 ($[M+H]^+$, 73%), 319.0 (M^+, 100%). HRMS (CI): calcd for $C_{16}H_{15}O_3NFS$ 320.0751 $[M+H]^+$, found 320.0729.

4.17.11. 6-Fluoro-2-(5-hydroxy-3,4-dimethoxyphenyl)-benzothiazole (11m). The product was obtained from **10b** and 5-hydroxy-3,4-dimethoxybenzaldehyde as a yellow solid in 69% yield, mp 174–175 °C. ^{1}H NMR ($CDCl_3$) δ: 7.98 (dd, $J = 4.5$, 8.9 Hz, 1H, H-4), 7.57 (dd, $J = 2.2$, 8.0 Hz, 1H, H-7), 7.31 (d, $J = 10.4$ Hz, 1H, H-2′), 7.25–7.18 (m, 2H, H-6′, H-5), 5.96 (br s, 1H, OH), 3.99 (s, 3H, OCH_3), 3.98 (s, 3H, OCH_3). LRMS (CI): 306.0 ($[M+H]^+$, 88%), 305.0 (M^+, 100%). HRMS (CI): calcd for $C_{15}H_{13}O_3NFS$ 306.0595 $[M+H]^+$, found 306.0585.

4.17.12. 6-Fluoro-2-(4-hydroxy-3,5-dimethoxyphenyl)-benzothiazole (11n). The product was obtained from **10b** and 4-hydroxy-3,5-dimethoxybenzaldehyde as a yellow solid in 78% yield, mp 173–174 °C. ^{1}H NMR ($CDCl_3$) δ: 7.79 (dd, $J = 5.1$, 8.8 Hz, 1H, H-4), 7.71 (dd, $J = 2.9$, 9.5Hz, 1H, H-7), 7.33 (s, 2H, H-2′, H-6′), 7.14 (dt, $J = 2.2$, 8.9 Hz, 1H, H-5), 5.88 (br s, 1H, OH), 4.01 (s, 6H, OCH_3). LRMS (CI): 306.0 ($[M+H]^+$, 81%), 305.0 (M^+, 100%). HRMS (CI): calcd for $C_{15}H_{13}O_3NFS$ 306.0595 $[M+H]^+$, found 306.0584.

4.18. General method for the preparation of carbon-11 labeled 4-, 5-, and 6-fluorinated 2-arylbenzothiazoles (3-[^{11}C]6b, 4-[^{11}C]11c, 3-[^{11}C]11c, 5-[^{11}C]11f, 4-[^{11}C]11f, 4-[^{11}C]11i, 3-[^{11}C]11i, 5-[^{11}C]11l, and 4-[^{11}C]11l)

$^{11}CO_2$ was produced by the $^{14}N(p,\alpha)^{11}C$ nuclear reaction in small volume (9.5 cm^3) aluminum gas target (CTI)

from 11 MeV proton cyclotron on research purity nitrogen (+1% O_2) in a Siemens radionuclide delivery system (Eclipse RDS-111). The phenolic hydroxyl precursor **7**, **11d**, **11e**, **11g**, **11h**, **11j**, **11k**, **11m** or **11n** (0.1–0.3 mg) was dissolved in CH_3CN (300 μL). To this solution was added 3 N NaOH (2–3 μL). The mixture was transferred to a small reaction vial. $^{11}CH_3OTf$ that was produced by the gas-phase production method[19] from $^{11}CO_2$ through $^{11}CH_4$ and $^{11}CH_3Br$ was passed into the reaction vial, which was cooled to 0 °C, until radioactivity reached a maximum (~2 min), and then the reaction vial was isolated and heated at 80 °C for 3 min. The contents of the reaction vial were diluted with $NaHCO_3$ (1 mL, 0.1 M). This solution was passed onto a C-18 cartridge by gas pressure. The cartridge was washed with H_2O (2× 3 mL), and the aqueous washing was discarded. The product was eluted from the column with EtOH (2× 3 mL) and then passed onto a rotatory evaporator. The solvent was removed by evaporation under vacuum. The labeled product 3-[^{11}C]**6b**, 4-[^{11}C]**11c**, 3-[^{11}C]**11c**, 5-[^{11}C]**11f**, 4-[^{11}C]**11f**, 4-[^{11}C]**11i**, 3-[^{11}C]**11i**, 5-[^{11}C]**11l**, or 4-[^{11}C]**11l** was formulated with saline, whose volume was dependent upon the use of the labeled product in tissue biodistribution studies (~6 mL, 3× 2 mL) or in PET imaging studies (1–3 mL) of tumor-bearing athymic mice,[24] sterile-filtered through a sterile vented Millex-GS 0.22 μm cellulose acetate membrane, and collected into a sterile vial. Total radioactivity was assayed and total volume was noted. The overall synthesis, purification, and formulation time was ~20 min from EOB. The decay corrected yield from $^{11}CO_2$ was 30–55%. Retention times in the analytical HPLC system were: t_R(**7**) = 3.39 min, t_R(3-[^{11}C]**6b**) = 4.51 min; t_R(**11d**) = 3.63 min, t_R(4-[^{11}C]**11c**) = 4.74 min; t_R(**11e**) = 3.52 min, t_R(3-[^{11}C]**11c**) = 4.74 min; t_R(**11g**) = 3.62 min, t_R(5-[^{11}C]**11f**) = 4.87 min; t_R(**11h**) = 3.42 min, t_R(4-[^{11}C]**11f**) = 4.87 min; t_R(**11j**) = 3.65 min, t_R(4-[^{11}C]**11i**) = 4.77 min; t_R(**11k**) = 3.55 min, t_R(3-[^{11}C]**11i**) = 4.77 min; t_R(**11m**) = 3.62 min, t_R(5-[^{11}C]**11l**) = 4.87 min; t_R(**11n**) = 3.43 min, t_R(4-[^{11}C]**11l**) = 4.87 min.

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