SUPPLEMENTAL INFORMATION

Imaging Caspase-3 Activation as a Marker of Apoptosis-Targeted Treatment Response in Cancer

Molecular Imaging and Biology

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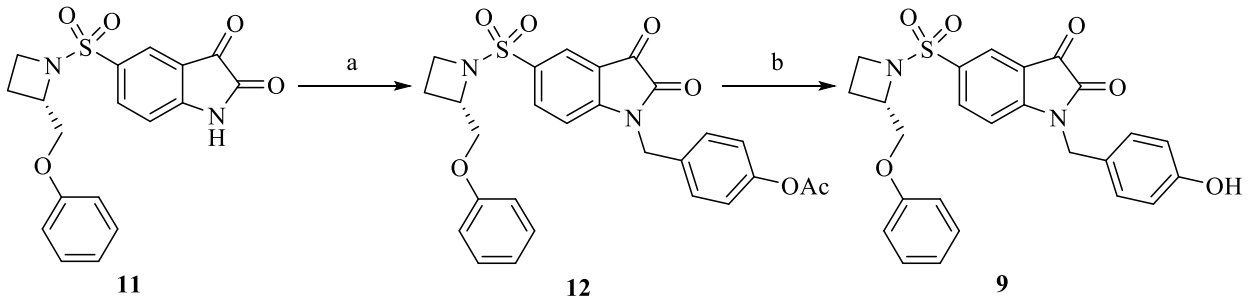
RADIOLABELING AND PURIFICATION

Precursor Synthesis Scheme:



Reagents: (a) 1) NaH, DMF, 2) $ClCH_2C_6H_4OAc$; (b) NaOH, CH_3OH, H_2O.

Scheme S1. Synthesis of precursor **9** in Figure 1 of the manuscript.

Chemistry. The synthesis of precursor **9** is shown in Scheme S1. The nitrogen of compound **11** was alkylated by treatment **11** with sodium hydride in DMF at 0 °C and the resulted salt was reacted with 4-(chloromethyl)phenyl acetate to give compounds **12** in 43% yield. The **12** was hydrolyzed with sodium hydroxide in methanol and water to afford **9**.

Detailed Precursor Synthesis Methods:

All reactions were carried out under an inert nitrogen atmosphere with dry solvents, under anhydrous conditions, unless otherwise stated. Reagents and grade solvents were used without further purification. Flash column chromatography was carried using Merck Kiesel gel 60 silica gel (230-400 mesh). ^{1}H NMR spectra were recorded at 300 MHz on a Varian Mercury-VX spectrometer. All chemical shift values are reported in ppm (δ).

(S)-4-((2,3-dioxo-5-((2-(phenoxymethyl)azetidin-1-yl)sulfonyl)indolin-1-yl)methyl)phenyl acetate (12) A solution of **11** (186 mg, 0.5 mmol) in DMF (3 mL) was added 60% NaH (30 mg, 0.75 mmol) at 0 °C. The mixture was stirred 15 min, then 4-(chloromethyl)phenyl acetate (0.5

mL) was added. The mixture was stirred overnight at rt, ether (75 mL) was added, washed with water (30 mL), saturated NaCl (30 mL) and dried over Na_2SO_4. After evaporation of the solvent, the crude product was purified by silica gel column chromatography eluting with hexane-ether (1:2) to afford 223 mg (43%) of **12** as a yellow solid, mp 145.3-146.4 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, *J* = 1.6 Hz, 1H), 7.94 (dd, *J* = 8.2 Hz, 1.6 Hz, 1H), 7.35 (d, *J* = 8.0 Hz, 2H), 7.22 (t, *J* = 8.0 Hz, 2H), 7.10 (d, *J* = 8.8 Hz, 2H), 6.93 (t, *J* = 7.6 Hz, 1H), 6.88 (d, *J* = 8.4 Hz, 1H), 6.79 (d, *J* = 8.0 Hz, 2H), 4.90 (s, 2H), 4.41 (m, 1H), 4.10 (m, 2H), 3.80 (t, *J* = 7.8 Hz, 2H), 2.30 (s, 3H), 2.36-2.19 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.4, 169.3, 158.1, 157.7, 153.3, 150.7, 137.8, 132.7, 131.3, 129.5, 128.8, 125.1, 122.4, 121.2, 117.4, 114.4, 111.1, 68.9, 62.1, 48.3, 43.8, 21.1, 19.0.

(S)-1-(4-hydroxybenzyl)-5-((2-(phenoxymethyl)azetidin-1-yl)sulfonyl)indoline-2,3-dione (9) A solution of **12** (130 mg, 0.25 mmol) in methanol (3 mL) and water (1 mL) was added NaOH (12 mg, 0.30 mmol) at rt. The mixture was stirred overnight, then acidified with 1 M HCl to pH=4, extracted with ethyl acetate (50 mL). The ethyl acetate was washed with NaCl (30 mL) and dried over Na_2SO_4. After evaporation of the solvent, the crude product was purified by silica gel column chromatography eluting with ether to afford 76 mg (64%) of **9** as a yellow solid, mp 120.6-121.3 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (s, 1H), 7.93 (d, *J* = 8.0 Hz, 1H), 7.20 (t, J = 8.0 Hz, 2H), 7.18 (d, *J* = 8.4 Hz, 2H), 6.92 (t, *J* = 7.6 Hz, 1H), 6.87 (d, *J* = 8.4 Hz, 1H), 6.82 (d, *J* = 8.4 Hz, 2H), 6.77 (d, *J* = 8.0 Hz, 2H), 5.65 (s, 1H), 4.82 (s, 2H), 4.42 (m, 1H), 4.08 (m, 2H), 3.81 (m, 2H), 2.33 (m, 1H), 2.22 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.6, 158.0, 157.8, 156.0, 153.6, 137.7, 132.6, 129.5, 129.2, 125.5, 125.0, 121.1, 117.4, 116.1, 114.4, 111.2, 68.8, 62.2, 48.3, 43.9.

General method of radiosynthesis of isatin analogues via Cu(I) catalyzed click chemistry using [^{18}F]fluoroethyl azide: [^{18}F]fluoroethyl azide (**2**) was radiosynthesized and isolated from the reaction mixture according to literature [1] with the modification of using t-amyl alcohol (200

μL) as the solvent. Into the tube containing 2 at room temperature were added alkyne precursor (~1.6 mg) in DMF (200 μL) and a yellowish mixture of CuSO4•5H2O (5 mg) and sodium ascorbate (15 mg) in water (100 μL). After 10 min at room temperature, the reaction mixture was acidified by 1 N HCl (1 mL) and then diluted in water (50 mL). The diluted solution passed through a Waters Oasis HLB cartridge (500 mg). The cartridge was rinsed with water (10 mL), and then acetonitrile (1 mL), methanol (1 mL), and water (2 mL) were used to elute the radioactivity for HPLC purification. Reversed phase HPLC was applied to purify the final product using an Alltech Econosil C18 250 × 10 mm 10 μ column with UV at 254 nm and flow rate at 4 mL/min. The HPLC purified final product was collected and the final dose was prepared using standard solid phase extraction (SPE) method (Waters C18 Seppak) in 10 % ethanol/saline solution. The final dose was analyzed by an analytical HPLC using an Alltech Altima C18 250 × 4.6 mm 10 μ with UV at 254 nm and a flow at 1 mL/min. The following mobile phases were used for purification and analysis: 2 : 1 MeOH/MeCN (**A**), ammonium formate solution (0.1 M, pH = 6.5) (**B**), MeOH (**C**), and ammonium formate buffer (0.1 M, pH = 4.5) (**D**).

WC-4-116: Semi-preparative HPLC: mobile phase (45 % **A**/55 % **B**), retention time: 23 min; analytical HPLC: mobile phase (60 % **A**/40 % **D**), retention time: 5.1 min; radiochemical yield: 38.9 ± 10.4 % (n = 4); radiochemical purity: > 99 %; specific activity: 800 ± 577 mCi/μmol (n = 4) at the end of synthesis.

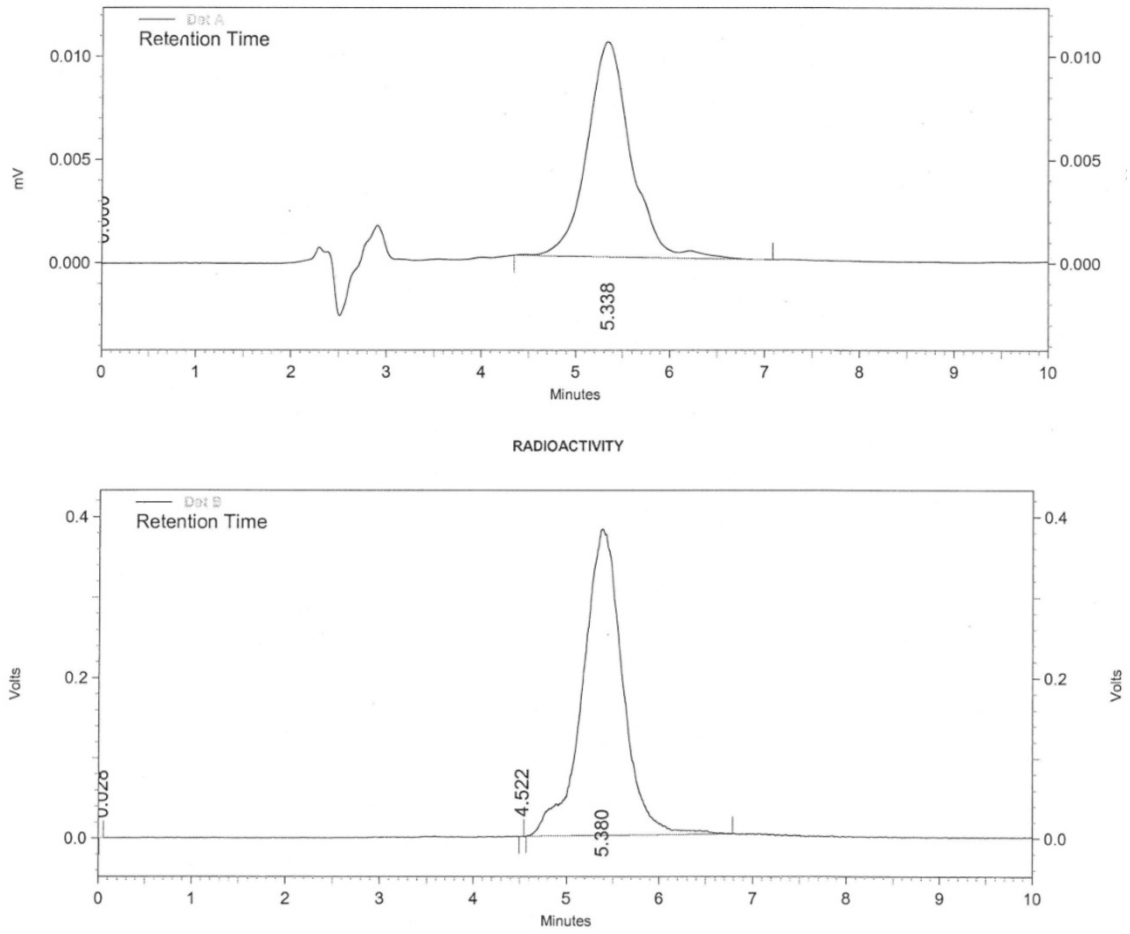


Figure S1. Analytical HPLC of [^{18}F]WC-4-116 with co-injection of reference: Top (UV); Bottom (radioactivity).

WC-4-122: Semi-preparative HPLC: mobile phase (45 % **A**/55 % **B**), retention time: 13 min; analytical HPLC: mobile phase (50 % **C**/50 % **D**), retention time: 6.3 min; radiochemical yield: 48 %; radiochemical purity: 98.7 %; specific activity: 300 mCi/μmol at the end of synthesis.

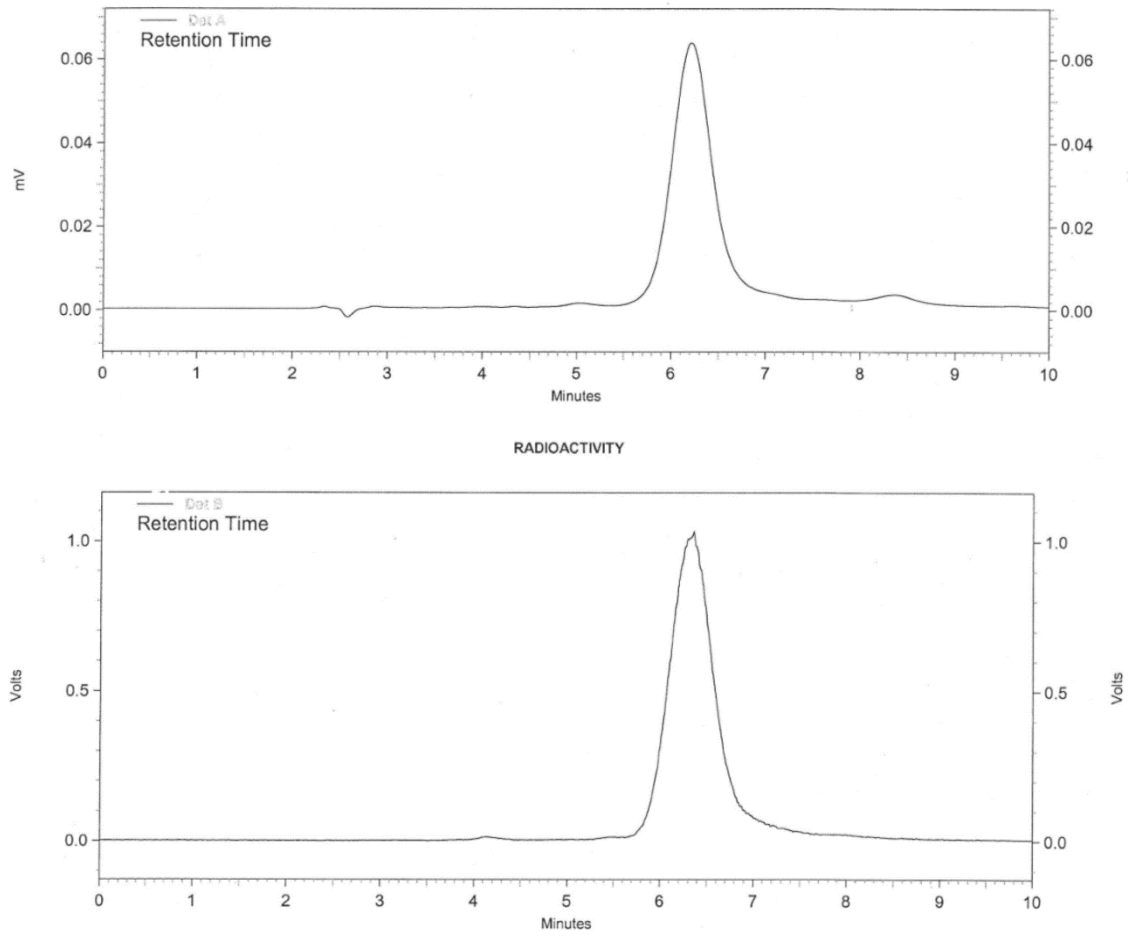


Figure S2. Analytical HPLC of [^{18}F]WC-4-122 with co-injection of reference: Top (UV); Bottom (radioactivity).

WC-4-131: Semi-preparative HPLC: mobile phase (45 % **A**/55 % **B**), retention time: 23 min; analytical HPLC: mobile phase (70 % **A**/30 % **D**), retention time: 4.6 min; radiochemical yield: 33.5 %; radiochemical purity: 99.3 %; specific activity: 443 mCi/μmol at the end of synthesis.

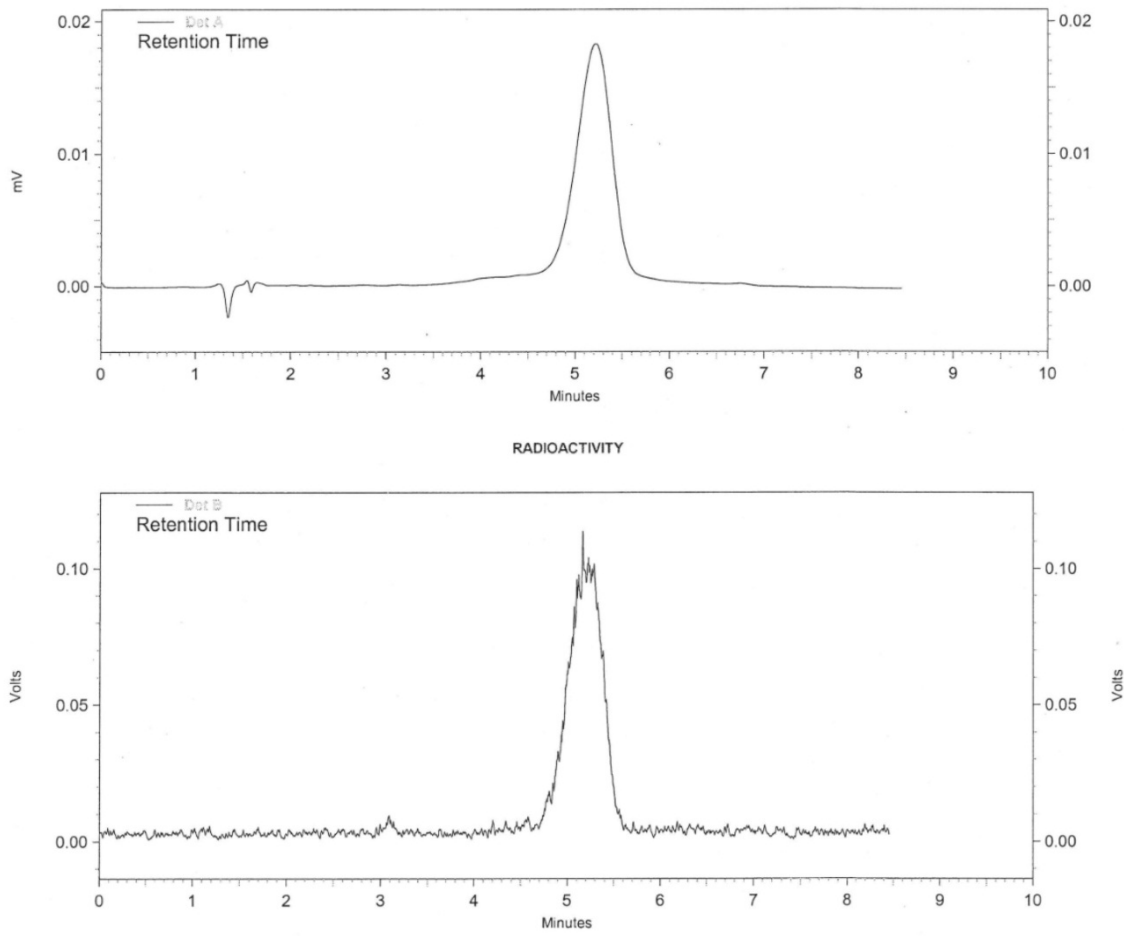


Figure S3. Analytical HPLC of [^{18}F]WC-4-131 with co-injection of reference: Top (UV); Bottom (radioactivity).

ICMT-18: Semi-preparative HPLC: mobile phase (34 % **A**/66 % **B**), retention time: 14 min; analytical HPLC: mobile phase (46 % **A**/54 % **B**), retention time: 5.4 min; radiochemical yield: 20.2 %; radiochemical purity: 99.9 %; specific activity: 652 mCi/μmol at the end of synthesis.

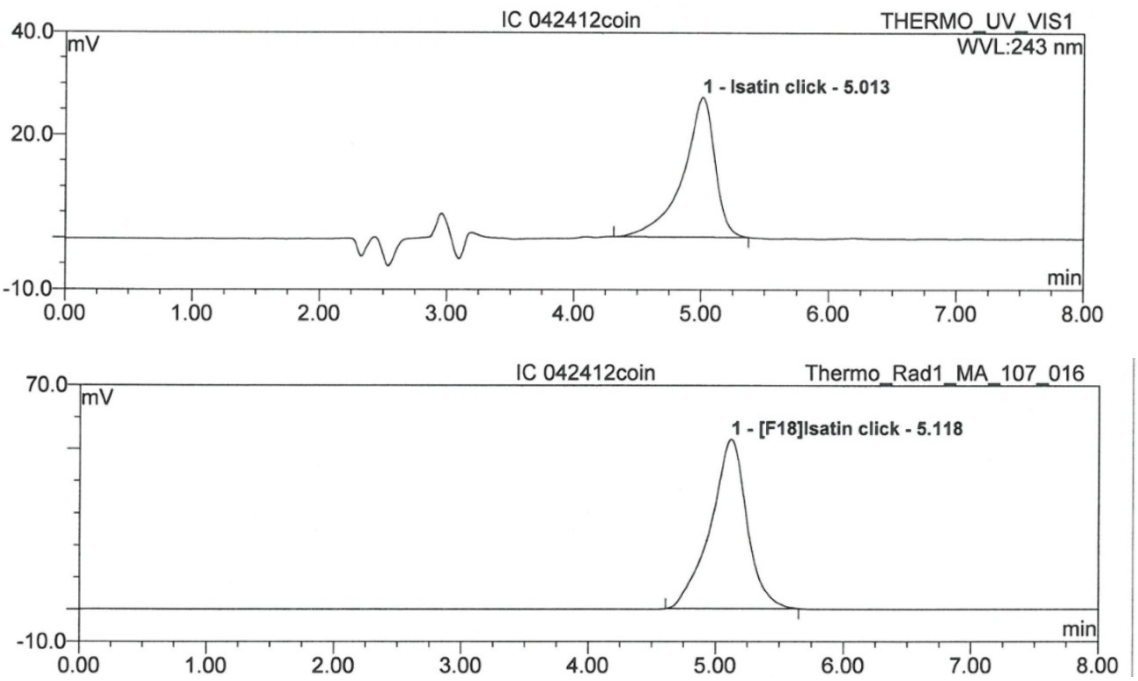


Figure S4. Analytical HPLC of [^{18}F]ICMT-18 with co-injection of reference: Top (UV); Bottom (radioactivity).

Radiosynthesis of 4: Ditosylate precursor **3** (5 mg) in acetonitrile (0.5 mL) was added to [^{18}F]fluoride (159 mCi), azeotropically dried with acetonitrile (3 mL) at 105 °C in the presence of K_2CO_3 (1 mg) and K_{2222} (5.6 mg). The reaction mixture was heated at 105 °C for 8 min. At room temperature, acetonitrile (1 mL) and water (3 mL) were added for HPLC injection. A reversed phase HPLC was applied to purify **4** using a Grace Econosphere C18 250 × 10 mm 10 μ column and 35 % MeCN/65 % ammonium formate buffer (0.1 M, pH = 4.5) as mobile phase with UV at 254 nm and flow rate at 4 mL/min. **4** (52 mCi) was collected at 17 min, and then diluted in water (50 mL). The diluted solution passed through a C18 Seppak (Waters, Classic), and then the Seppak was rinsed with water (10 mL), dried by a flow of N_2. **4** was eluted from the Seppak with dichloromethane (3 × 1 mL), dried by a Na_2SO_4 cartridge, and collected in a 10 mL tube. Dichloromethane was removed under a flow of N_2 at 105 °C to afford **4** (37 mCi).

Radiosythesis of [^{18}F]WC-4-35: Compound **5** (0.2 mg) in MeCN (100 μL) was treated with 1 N Bu_4NOH (0.83 μL) in MeCN (200 μL), and then the mixture was added to the tube containing **4**. The mixture was heated at 105 °C for 10 min, followed by addition of 1 N HCl (200 μL) and heating at 105 °C for 5 min. At room temperature, MeCN (1 mL), MeOH (1 mL) and ammonium formate solution (0.1 M, pH = 6.5) were added for HPLC injection. Reversed phase HPLC was applied to purify the final product using an Alltech Econosil C18 250 × 10 mm 10 μ column and 70 % 2 : 1 MeOH/MeCN and 30 % ammonium formate solution (0.1 M, pH = 6.5) as mobile phase with UV at 254 nm and flow rate at 4 mL/min. **4** (12.9 mCi) was collected at 13.5-15.5 min. Standard SPE method was used to prepare the dose of **4** in 15 % ehanol/saline. The dose of **4** was analzed by a reversed phase analytical HPLC using Alltech Altima C18 250 × 4.6 mm 10 μ column and 75 % MeOH/25% ammonium formate solution (0.1 M, pH = 6.5) as mobile phase with UV at 254 nm and flow rate at 1.5 mL/min. The retention time of **4** was 4.5 min. Radiochemical purity is 99.9 %. The specific activity at the end of synthesis is 996 mCi/μmol.

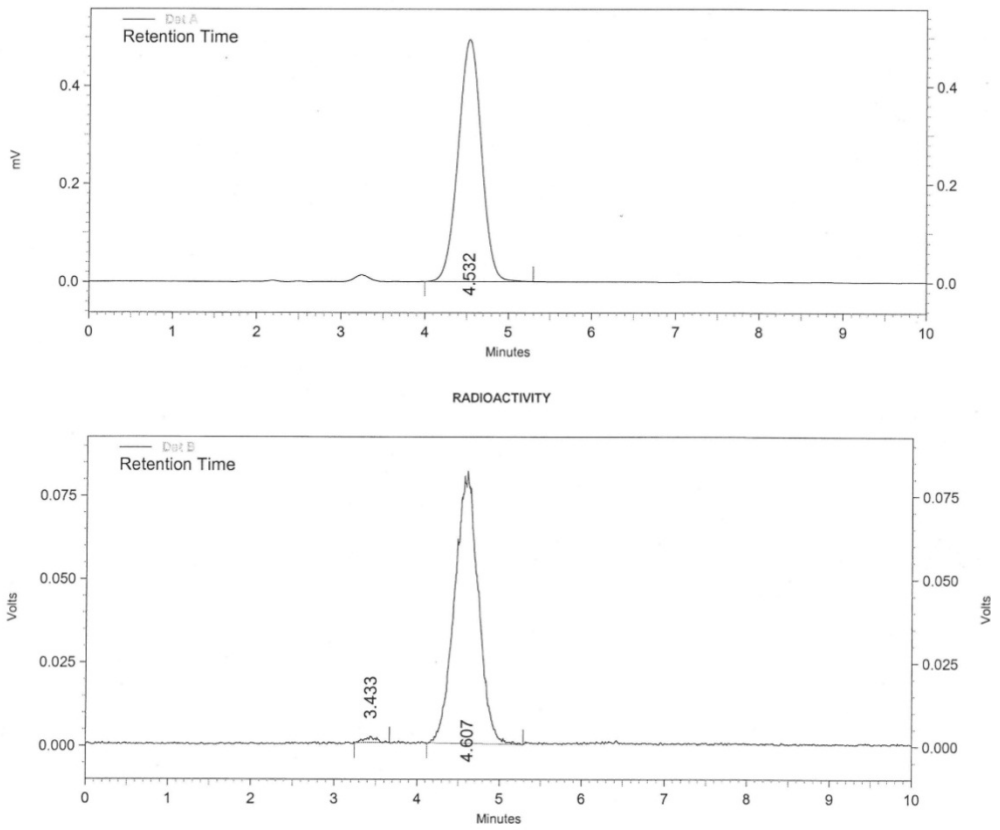


Figure S5. Analytical HPLC of [^{18}F]WC-4-35 with co-injection of reference: Top (UV); Bottom (radioactivity).

Radiosynthesis of [^{18}F]WC-4-36: The same procedure as that for **[^{18}F]WC-4-35** was used to radiosynthesize **[^{18}F]WC-4-35**, starting from precursor **5**. The radiochemical purity was 99.7 %, and the specific activity at the end of synthesis was 621 mCi/μmol.

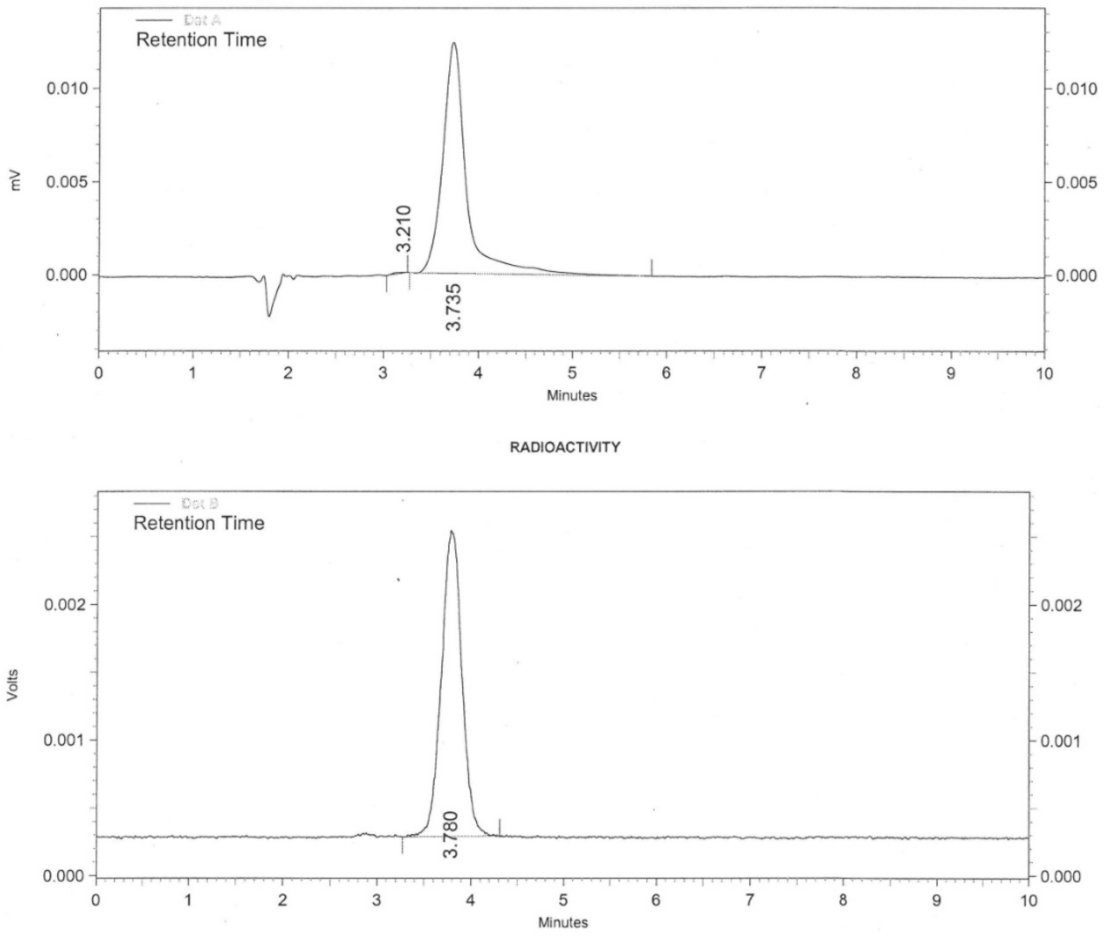


Figure S6. Analytical HPLC of [^{18}F]WC-4-36 with co-injection of reference: Top (UV); Bottom (radioactivity).

Supplement References

1. Zhou D, Chu W, Dence CS, Mach RH, Welch MJ (2012) Highly efficient click labeling using 2-[(1)(8)F]fluoroethyl azide and synthesis of an (1)(8)FN-hydroxysuccinimide ester as conjugation agent. Nucl Med Biol 39:1175-1181.