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Synthesis of (*Z*)-2-((1*H*-indazol-3-yl)methylene)-6-[^{11}C] methoxy-7-(piperazin-1-ylmethyl)benzofuran-3(2*H*)-one as a new potential PET probe for imaging of the enzyme PIM1

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Proviral integration site in moloney murine leukemia virus kinase 1 (PIM1)
Radiosynthesis
Positron emission tomography (PET)
Alzheimer's disease
Cancer

ABSTRACT

(*Z*)-2-((1*H*-Indazol-3-yl)methylene)-6-methoxy-7-(piperazin-1-ylmethyl)benzofuran-3(2*H*)-one is a potent and selective proviral integration site in moloney murine leukemia virus kinase 1 (PIM1) inhibitor with an IC_{50} value of 3 nM. (*Z*)-2-((1*H*-Indazol-3-yl)methylene)-6-[^{11}C]methoxy-7-(piperazin-1-ylmethyl) benzofuran-3(2*H*)-one, a new potential PET probe for imaging of the enzyme PIM1, was first designed and synthesized in 20–30% decay corrected radiochemical yield and 370–740 GBq/μmol specific activity at end of bombardment (EOB). The synthetic strategy was to prepare a carbon-11-labeled Boc-protected intermediate followed by a quick acidic de-protection.

Protein kinase (PK) is a family of enzymes that are involved in controlling the function of other proteins through the phosphorylation of hydroxyl groups of serine and threonine and/or tyrosine amino acid residues on these proteins.[1] Most PKs act on both serine and threonine (serine/threonine kinases), others act on tyrosine (tyrosine kinases), and a number act on all three (dual-specificity kinases), and these PKs regulate all the critical phases of cell growth including cell proliferation, differentiation, and apoptosis.[1] Proviral integration site in moloney murine leukemia virus kinase 1 (PIM1) is a member of serine/threonine kinase.[2] PK expression and activity are associated with various disease processes, such as Alzheimer's disease and cancer.[3] In cancer, PIM1 phosphorylate multiple proteins as well as drug efflux transporters, resulting in drug resistance.[2] Overexpression of PIM1 in cancers indicates PIM1 is an emerging target for cancer therapy.[4,5] PIM1 is an attractive target for cancer imaging as well, however, so far no specific cancer imaging agent is developed for PIM1.[6] We are interested in development of new cancer imaging agents for the biomedical imaging technique positron emission tomography (PET) to image cancer and to monitor the response of cancer to therapy. In our previous work, we have developed a series of radiolabeled (carbon-11 and fluorine-18) PK inhibitors such as [^{11}C]MKC-1 ([^{11}C]Ro 31-7453), [^{11}C]SB-216763, [^{11}C]Enzastaurin ([^{11}C]LY317615), [^{11}C]GSK2126458, 2-[^{18}F]GSK 2126458, 4-[^{18}F]GSK2126458, [^{11}C]Gefinitib ([^{11}C]Iressa), *N*-[^{11}C] Vandetanib and *O*-[^{11}C]Vandetanib, as shown in Figure 1, as new cancer imaging agents for PET to study PK and PK inhibitors.[7–12] In this ongoing study, we target PIM1. (*Z*)-2-((1*H*-Indazol-3-yl)methylene)-6-methoxy-7-(piperazin-1-ylmethyl)benzofuran-3(2*H*)-one (**4**) is a potent and selective PIM1 inhibitor with an IC_{50} value of 3 nM, recently developed by Nakano et al.[2] To radiolabel therapeutic agents as diagnostic agents for imaging of PK and monitoring of therapeutic efficacy of PK inhibitors, we have designed and synthesized (*Z*)-2-((1*H*-indazol-3-yl)methylene)-6-[^{11}C]methoxy-7-(piperazin-1-ylmethyl)benzofuran-3(2*H*)-one ([^{11}C]**4**) as a new potential PET probe, for the first time.

The target compound **4**, and its Boc-protected precursor **5** and intermediate **3** were synthesized by adopting the available literature methods from previous work.[2] As illustrated in Scheme 1, Mannich reaction of 6-hydroxybenzofuran-3(2*H*)-one and *tert*-butyl piperazine-1-carboxylate with paraformaldehyde in EtOH at reflux provided compound **1** in 62% yield. The yield was improved by eliminating microwave irradiation. Mitsunobu coupling reaction of **1** and MeOH with diisopropyl azodicarboxylate

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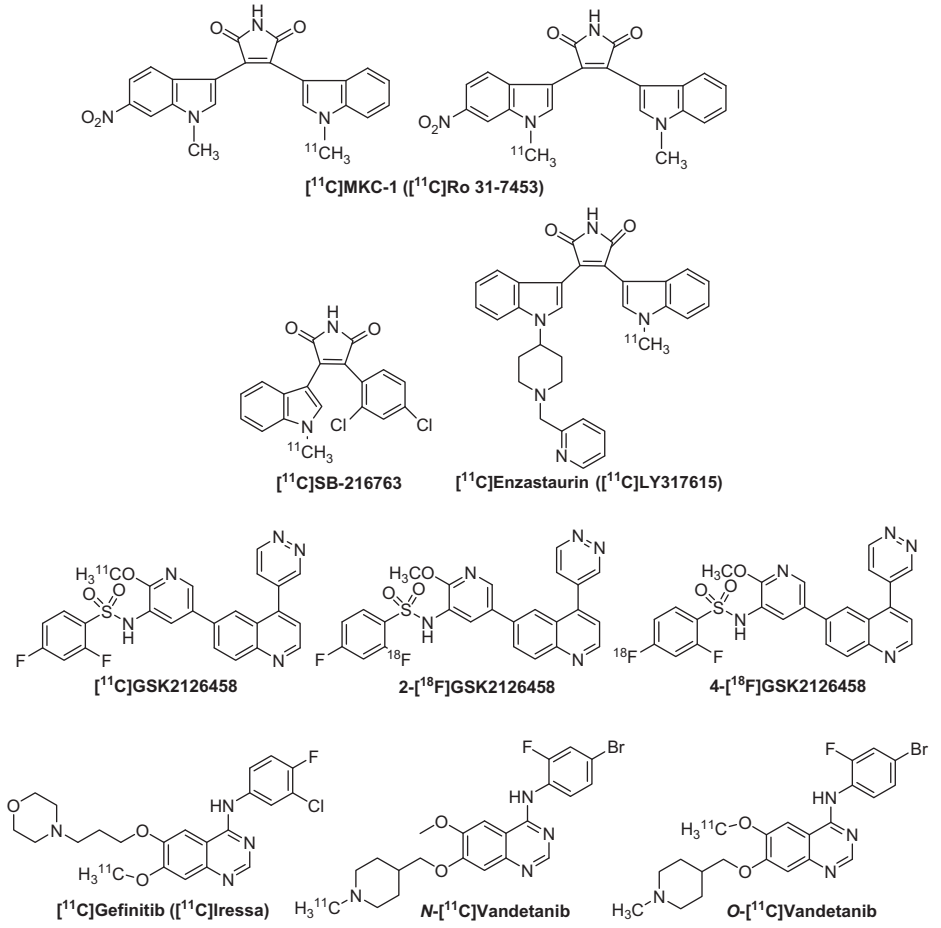
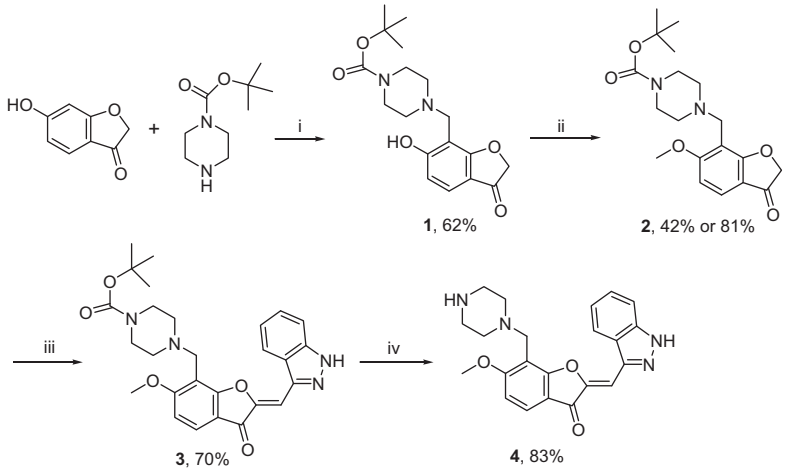


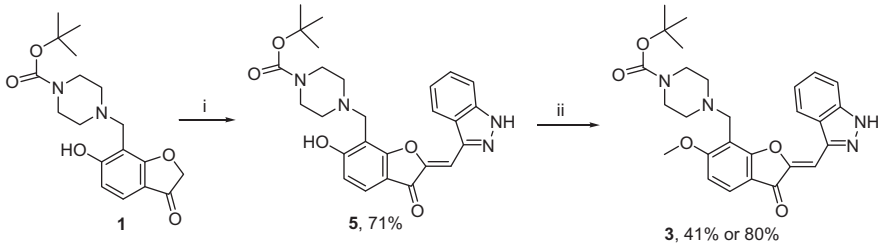
Figure 1. Radiolabeled PK inhibitors.



Scheme 1. Synthesis of reference standard **4** and Boc-protected intermediate **3**. Reagents and conditions: (i) paraformaldehyde, EtOH, reflux; (ii) MeOH, DIAD, PPh_3, THF, room temperature (RT); or CH_2N_2, THF, RT; (iii) 1*H*-indazole-3-carbaldehyde, piperidine, MeOH, reflux; (iv) TFA, CH_2Cl_2, RT.

(DIAD) and triphenylphosphine as catalysts afforded **2** in 42% yield. This methylation method gave low yield, because it generated a few side products and it was difficult to work up. Alternative methylation method of **1** with diazomethane[13] was adopted to improve the yield, which afforded **2** in 81% yield. Another methylation method using typical methylating agent CH_3I was tested as well. However, it gave poor chemical yield. Knoevenagel condensation of **2** with 1*H*-indazole-3-carbaldehyde provided **3** in 70% yield. Subsequent acidic removal of Boc group using trifluoroacetic acid (TFA) obtained reference standard **4** in 83% yield.

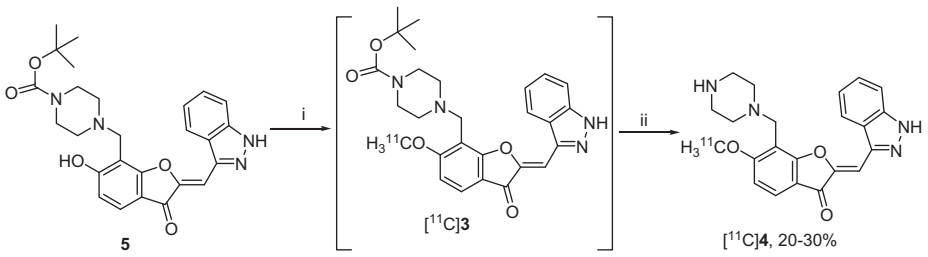
As outlined in Scheme 2, a new synthetic route was designed to synthesize new compound Boc-protected precursor **5** and intermediate **3**. First Knoevenagel condensation of **1** with 1*H*-indazole-3-carbaldehyde gave precursor **5** in 71% yield, then methylation of **5** in two different ways aforementioned furnished **3** in 41% and 80% yield, respectively.



Scheme 2. Synthesis of Boc-protected precursor **5** and Boc-protected intermediate **3**. Reagents and conditions: (i) 1*H*-indazole-3-carbaldehyde, piperidine, MeOH, reflux; (ii) MeOH, DIAD, PPh_3, THF, RT; or CH_2N_2, THF, RT.

Synthesis of the target radiotracer [^{11}C]**4** is indicated in Scheme 3. Boc-protected precursor **5** was labeled by [^{11}C]methyl triflate ([^{11}C]CH_3OTf) prepared from [^{11}C]CO_2,[14,15] in the presence of 2 N NaOH in acetonitrile through the *O*-[^{11}C]methylation[10–12] to provide a radiolabeling Boc-protected intermediate [^{11}C]**3**. Without further purification, [^{11}C]**3** was quickly converted to the target tracer [^{11}C]**4** by acidic removal of Boc group under 1 N HCl. [^{11}C]**4** was isolated by a semi-preparative high performance liquid chromatography (HPLC) with a Prodigy C-18 column from Phenomenex and a solid-phase extraction (SPE) with a disposable C-18 Plus Sep-Pak cartridge from Waters (a second purification or isolation process)[10,16,17] to produce the pure radiolabeled compound in 20–30% radiochemical yield, decay corrected to end of bombardment (EOB), based on [^{11}C]CO_2. The radiolabeling yield was relatively low, since it was a one-pot two-step radiolabeling reaction, but it was similar to the values previously reported by our lab for the tracers synthesized from the same synthetic strategy.[7–9] Addition of $NaHCO_3$ to quench the radiolabeling reaction and to dilute the radiolabeling mixture prior to the injection onto the semi-preparative HPLC column for purification gave better separation of [^{11}C]**4** from its radiolabeled intermediate [^{11}C]**3** and normethyl precursor **5**.[10,16,17] The radiosynthesis was performed in a home-built automated multi-purpose ^{11}C-radiosynthesis module, allowing measurement of specific radioactivity during synthesis.[18–20] This ^{11}C-radiosynthesis module includes the overall design of the reaction, purification and reformulation capabilities of the prototype system. In addition, the specific activity (SA, GBq/μmol at EOB) of ^{11}C-tracer can be automatically determined prior to product delivery for compounds purified by the HPLC-portion of the system.[21] Briefly, analysis of the chromatographic data utilized PeakSimple software (SRI Instruments, Las Vegas, NV, USA). Immediately following elution of the product peak, the chromatographic data are exported to PeakSimple readable files, and the area of the radioactivity peak is converted to GBq at EOB by comparison to a reference calibration curve previously constructed using the same detector, loop and flow rate. The mass peak from the UV chromatogram (without decay correction) is similarly compared to a standard curve made at the same UV wavelength, mobile phase and flow rate. Simple division of the total EOB radioactivity peak (in GBq) by the total mass peak (in nmoles) gives SA at EOB in GBq/μmol. The overall synthesis, purification and reformulation time was 30–40 min from EOB. The SA was in a range of 370–740 GBq/μmol at EOB. The SA can also be measured by analytical HPLC, which is consistent with the on-the-fly technique to determine the SA by semi-preparative HPLC. Chemical purity and radiochemical purity were determined by analytical HPLC.[22] The chemical purity of the precursor **5**, intermediate **3** and reference standard **4** was >95%. The radiochemical purity of the target tracer [^{11}C]**4** was >99% determined by radio-HPLC through γ-ray (PIN diode) flow detector, and the chemical purity of the target tracers [^{11}C]**4** was >90% determined by reversed-phase HPLC through UV flow detector.

SA is defined as the radioactivity per unit mass of a radionuclide or a labeled compound. SA (MBq/mg) = $3.13 \times 10^9/A \times t_{1/2}$, where A is the mass number of the radionuclide, and $t_{1/2}$ is the half-life in hours of the radionuclide. For carbon-11, carrier-free ^{11}C, maximum (theoretical) ^{11}C SA = 340,918 GBq/μmol.[23] Actual SAs of the ^{11}C-tracers in the PET chemistry facility are depended on two parts: (1) carrier from the ^{11}C-target, and (2) carrier from the ^{11}C-radiosynthesis unit.[24,25] Furthermore, actual SA for the ^{11}C-tracers synthesized by ^{11}C-methylation with [^{11}C]CH_3OTf in our PET chemistry facility is depended on two parts: (1) carrier from the cyclotron consisted of the ^{11}C gas irradiation target system, and (2) carrier from the [^{11}C]methyl triflate ([^{11}C]CH_3OTf) system, ^{11}C radiolabeled precursor or called ^{11}C radiolabeled methylating reagent. The ^{11}C gas target we used is the Siemens RDS-111 Eclipse cyclotron ^{11}C gas target. The [^{11}C]CH_3OTf production system we used is an Eckert & Ziegler Modular Lab C-11 Methyl Iodide/Triflate module, convenient gas phase bromination of [^{11}C]methane and production of [^{11}C]CH_3OTf, a 'dry' method using Br_2 different with other 'dry' method using I_2 and 'wet' method using $LiAlH_4$ and HI. Our system will have much less ^{12}C carrier-added in comparison with other 'dry' method and 'wet' method.[15] The SA for our ^{11}C-tracers usually ranges from 370 to 925 GBq/μmol at EOB according to our previous works. Our study has proved that the maximum in-target ^{11}C SA is ~925 GBq/μmol (25 Ci/μmol) at EOB in our cyclotron and [^{11}C]CH_3OTf system. The SA of the title tracer was 370–740 GBq/μmol at EOB, which was similar to the values previously reported by our lab. A historical perspective on the SA of radiopharmaceuticals indicated it is difficult to achieve high SA for the ^{11}C-tracers.[23] However, a recent paper reported the maximum in-target ^{11}C SA 2775 GBq/μmol (75 Ci/μmol) at EOB has been obtained, using GE



Scheme 3. Synthesis of target tracer [^{11}C]**4**. Reagents and conditions: (i) [^{11}C]CH_3OTf, CH_3CN, 2 N NaOH, 80 °C, 3 min; (ii) 1 N HCl, 80 °C, 3 min.

PETtrace cyclotron and TRACERLab FXC automated synthesizer for the synthesis of a ^{11}C-tracer, [^{11}C]GR103545.[26]

The experimental details and characterization data for compounds **1–5** and for the tracer [^{11}C]**4** are given.[27]

In summary, a carbon-11-labeled PIM1 inhibitor (*Z*)-2-((1*H*-indazol-3-yl)methylene)-6-[^{11}C]methoxy-7-(piperazin-1-ylmethyl)benzofuran-3(2*H*)-one ([^{11}C]**4**) was first designed and synthesized as a new potential PET probe for imaging of the enzyme PIM1. An automated multi-purpose ^{11}C-radiosynthesis module of our own design for fully automated synthesis of [^{11}C]**4** has been built, featuring the measurement of specific activity by the on-the-fly technique. The radiosynthesis employed a one-pot two-step reaction via *O*-[^{11}C]methylation radiolabeling on hydroxyl group of the Boc-protected phenolic precursor followed by a quick acidic removal of Boc group, incorporated efficiently with the most commonly used [^{11}C]methylating agent, [^{11}C]CH_3OTf, produced by gas-phase production of [^{11}C]methyl bromide ([^{11}C]CH_3Br) from our laboratory. The target tracer was isolated and purified by a semi-preparative HPLC procedure in moderate radiochemical yields, short overall synthesis time, and high specific activity. These results facilitate the potential preclinical and clinical PET studies of radiolabeled PIM1 inhibitor [^{11}C]**4** in animals and humans.

Acknowledgments

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27. (a) General. All commercial reagents and solvents were purchased from Sigma–Aldrich and Fisher Scientific, and used without further purification. [^{11}C]CH_3OTf was prepared according to a literature procedure.[15] Melting points were determined on a MEL-TEMP II capillary tube apparatus and were uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded at 500 and 125 MHz, respectively, on a Bruker Avance II 500 MHz 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*) were reported in hertz (Hz). LC–MS analysis was performed on an Agilent system, consisting of an 1100 series HPLC connected to a diode array detector and a 1946D mass spectrometer configured for positive-ion/negative-ion electrospray ionization. The high resolution mass spectra (HRMS) were obtained using a Waters/Micromass LCT Classic spectrometer. Chromatographic solvent proportions are indicated as volume: volume ratio. Thin-layer chromatography (TLC) was run using Analtech silica gel GF uniplates (5×10 cm^2). Plates were visualized under UV light. Normal phase flash column 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 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; mobile phase 3:1:3 CH_3CN/MeOH 20 mM phosphate buffer solution (pH 6.7); flow rate 1.5 mL/min; and UV (254 nm) and γ-ray (PIN diode) flow detectors. Semi-preparative HPLC was performed using a Prodigy (Phenomenex) 5 μm C-18 column, 12 nm, 10×250 mm; mobile phase 40% CH_3CN/60% 0.1 M NH_4OAc (pH 6.7); flow rate 5.0 mL/min; UV (254 nm) and γ-ray (PIN diode) flow detectors. C18 Plus Sep-Pak cartridges were obtained from Waters Corporation (Milford, MA). Sterile Millex-FG 0.2 μm filter units were obtained from Millipore Corporation (Bedford, MA).
(b) *tert*-Butyl 4-((6-hydroxy-3-oxo-2,3-dihydrobenzofuran-7-yl)methyl) piperazine-1-carboxylate (**1**). A mixture of 6-hydroxybenzofuran-3(2*H*)-one (14.82 g, 98.7 mmol), *tert*-butyl piperazine-1-carboxylate (18.39 g, 98.7 mmol) and paraformaldehyde (3.05 g, 101.66 mmol) in EtOH (150 mL) was heated to reflux for 5 h. The mixture was evaporated under reduced pressure, and the resulting residue was purified by column chromatography (10–100% EtOAc/hexanes) on silica gel to afford **1** (21.29 g, 62%) as a white solid; R_f = 0.70 (5% MeOH/CH_2Cl_2); mp 123–125 °C. ^{1}H NMR (DMSO-d_6): δ 1.38 (s, 9H, $3 \times CH_3$), 2.44 (t, J = 5.0 Hz, 4H, $2 \times CH_2$), 3.32 (br s, 4H, $2 \times CH_2$), 3.67 (s, 2H, CH_2), 4.73 (s, 2H, CH_2), 6.58 (d, J = 8.5 Hz, 1H, Ph–H), 7.39 (d, J = 8.5 Hz, Ph–H). MS (ESI): 349 ([M+H]$^+$, 100%).
(c) *tert*-Butyl 4-((6-methoxy-3-oxo-2,3-dihydrobenzofuran-7-yl)methyl) piperazine-1-carboxylate (**2**). Method A. Diisopropyl azodicarboxylate (3.03 g, 15.0 mmol) in THF (5.0 mL) was slowly added into a mixture of compound **1** (3.48 g, 10.0 mmol), MeOH (598 mg, 13.0 mmol), triphenylphosphine (3.93 g, 15.0 mmol) in THF (80 mL) at 0 °C. Then the reaction mixture was stirred at room temperature (RT) for 2 h, and concentrated in vacuo. The resulting residue was purified by column chromatography (0–3% MeOH/CH_2Cl_2) on silica gel to obtain **2** (1.52 g, 42%) as a white solid; R_f = 0.42 (5% MeOH/CH_2Cl_2); mp 68–70 °C. ^{1}H NMR ($CDCl_3$): δ 1.44 (s, 9H, $3 \times CH_3$), 2.48 (br s, 4H, $2 \times CH_2$), 3.42–3.43 (m, 4H, $2 \times CH_2$), 3.71 (s, 2H, CH_2), 3.93 (s, 3H, OCH_3), 4.64 (s, 2H, CH_2), 6.69 (d, J = 8.5 Hz, 1H, Ph–H), 7.61 (d, J = 8.5 Hz, 1H, Ph–H). MS (ESI): 363 ([M+H]$^+$, 100%). Method B. To a solution of compound **1** (696 mg, 2.0 mmol) in THF (50 mL), diazomethane (18 mmol) in ether was added at 0 °C, and the reaction mixture was stirred at RT for 1 h, and concentrated in vacuo. The resulting residue was purified through column chromatography (0–3% MeOH/CH_2Cl_2) on silica gel to afford **2** (586 mg, 81%) as a white solid. The analytical data were same as aforementioned.
(d) (*Z*)-*tert*-Butyl 4-((2-((1*H*-indazol-3-yl)methylene)-6-methoxy-3-oxo-2,3-dihydrobenzofuran-7-yl)methyl)piperazine-1-carboxylate (**3**). Method C for preparation of **3** from **2**. A mixture of compound **2** (1.81 g, 5.0 mmol), 1*H*-indazole-3-carbaldehyde (731 mg, 5.0 mmol), and piperidine (426 mg, 5.0 mmol) in MeOH (40 mL) was stirred at 60 °C for 3 h. Then the reaction was evaporated in reduced pressure, and the resulting residue was purified by column chromatography (0–8% MeOH/CH_2Cl_2) on silica gel to afford **3** (1.72 g, 70%) as a yellow solid; R_f = 0.40 (5% MeOH/CH_2Cl_2); mp 228–231 °C. ^{1}H NMR (DMSO-d_6): δ 1.37 (s, 9H. $3 \times CH_3$) 2.46 (t, J = 4.5 Hz, 4H, $2 \times CH_2$), 3.29 (br s, 4H, $2 \times CH_2$), 3.74 (s, 2H, CH_2), 3.97 (s, 3H, OCH_3), 7.05 (d, J = 8.5 Hz, 1H, Ph–H), 7.08 (s, 1H, CH=), 7.26 (t, J = 8.0 Hz, 1H, Ph–H), 7.47 (t, J = 8.0 Hz, 1H, Ph–H), 7.64 (d, J = 8.0 Hz, 1H, Ph–H), 7.79 (d, J = 8.5 Hz, 1H, Ph–H), 8.59 (d, J = 8.0 Hz, 1H, Ph–H), 13.86 (s, 1H, NH); ^{1}H NMR ($CDCl_3$): δ 1.42 (s, 9H, $3 \times CH_3$), 2.59 (br s, 4H, $2 \times CH_2$), 3.45 (br s, 4H, $2 \times CH_2$), 3.88 (s, 2H, CH_2), 3.97 (s, 3H, OCH_3), 6.81 (d, J = 8.5 Hz, 1H, Ph–H), 7.24–7.27 (m, 2H, Ph–H and CH=), 7.41 (dt, J = 0.5, 7.0 Hz, 1H, Ph–H), 7.52 (d, J = 8.0 Hz, 1H, Ph–H), 7.79 (d, J = 8.5 Hz, 1H, Ph–H), 8.39 (d, J = 7.5 Hz, 1H, Ph–H), 11.50 (br s, 1H, NH). MS (ESI): 491 ([M+H]$^+$, 100%); MS (ESI), 489 ([M−H]$^-$, 100%). Method D for preparation of **3** from **5**. The title compound was obtained in 41% and 80% yield, respectively, in the same manner as described for **2**. The analytical data were same as aforementioned.
(e) (*Z*)-2-((1*H*-Indazol-3-yl)methylene)-6-methoxy-7-(piperazin-1-ylmethyl) benzofuran-3(2*H*)-one (**4**). A mixture of compound **3** (0.49 g, 1.0 mmol) in the solution of TFA (2 mL) and CH_2Cl_2 (8 mL) was stirred at RT for 3 h. Then the mixture was evaporated under reduced pressure. The residue was added

aqueous $NaHCO_3$ to adjust pH to 8, and the mixture was extracted with CH_2Cl_2 (50 mL × 3), dried over Na_2SO_4, and concentrated. The crude was purified by column chromatography (5–20% MeOH/CH_2Cl_2) on silica gel to afford **4** (0.32 g, 83%) as a yellow solid; R_f = 0.12 (10% MeOH/CH_2Cl_2); mp >300 °C. ^{1}H NMR (DMSO-d_6): δ 2.50 (m, 4H, 2 × CH_2), 2.81 (br s, 4H, 2 × CH_2), 3.92 (s, 2H, CH_2), 3.99 (s, 3H, OCH_3), 7.10 (d, J = 8.5 Hz, 1H, Ph–H), 7.13 (s, 1H, CH=), 7.30 (t, J = 7.5 Hz, 1H, Ph–H), 7.47 (t, J = 7.5 Hz, 1H, Ph–H), 7.64 (d, J = 8.0 Hz, 1H, Ph–H), 7.84 (d, J = 8.5 Hz, 1H, Ph–H), 8.52 (d, J = 8.0 Hz, 1H, Ph–H), 13.87 (br s, 1H, NH). MS (ESI): 391 ([M+H]$^+$, 100%); MS (ESI): 389 ([M−H]$^-$, 100%).

(f) (*Z*)-*tert*-Butyl 4-((2-((1*H*-indazol-3-yl)methylene)-6-hydroxy-3-oxo-2,3-dihydrobenzofuran-7-yl)methyl)piperazine-1-carboxylate (**5**). The title compound was obtained in the same manner as described for **3** (Method C). From compound **1** (3.48 g, 10.0 mmol), 1*H*-indazole-3-carbaldehyde (1.46 g, 10.0 mmol), and piperidine (852 mg, 10.0 mmol), **5** (3.38 g, 71%) was obtained as a yellow solid; R_f = 0.43 (5% MeOH/CH_2Cl_2); mp >300 °C. ^{1}H NMR (DMSO-d_6): δ 1.37 (s, 9H, 3 × CH_3), 2.50–2.53 (m, 4H, 2 × CH_2), 3.34 (br s, 4H, 2 × CH_2), 3.82 (s, 2H, CH_2), 6.78 (d, J = 8.0 Hz, 1H, Ph–H), 7.05 (s, 1H, CH=), 7.27 (t, J = 7.5 Hz, 1H, Ph–H), 7.47 (t, J = 7.5 Hz 1H, Ph–H), 7.60 (d, J = 8.0 Hz, 1H, Ph–H), 7.63 (d, J = 8.0 Hz, 1H, Ph–H), 8.54 (d, J = 8.0 Hz, 1H, Ph–H), 13.82 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 5.99, 27.93, 50.09, 52.28, 78.74, 99.42, 106.83, 112.72, 121.42, 121.52, 121.64, 124.63, 126.66, 146.10, 153.62, 165.44, 166.34, 181.34. MS (ESI): 477 ([M+H]$^+$, 100%); MS (ESI): 475 ([M−H]$^-$, 100%). HRMS (ESI): calcd for $C_{26}H_{29}N_4O_5$, 477.2138 ([M+H]$^+$); found 477.2134.

(g) (*Z*)-2-((1*H*-Indazol-3-yl)methylene)-6-[^{11}C]methoxy-7-(piperazin-1-ylmethyl)benzofuran-3(2*H*)-one ([^{11}C]**4**). [^{11}C]CO_2 was produced by the ^{14}N(p,)^{11}C nuclear reaction in the small volume (9.5 cm^3) aluminum gas target provided with the Siemens RDS-111 Eclipse cyclotron. The target gas consisted of 1% oxygen in nitrogen purchased as a specialty gas from Praxair, Indianapolis, IN, USA. The proton-beam current was 55 μA, and the irradiation time was 30 min. The production run produced approximately 45.5 GBq of [^{11}C]CO_2 at EOB. The precursor **5** (0.1–0.3 mg) was dissolved in CH_3CN (300 μL). To this solution was added 2 N NaOH (3 μL). The mixture was transferred to a small reaction vial. No-carrier-added (high specific activity) [^{11}C]CH_3OTf that was produced by the gas-phase production method[15] from [^{11}C]CO_2 through [^{11}C]CH_4 and [^{11}C]CH_3Br with AgOTf column was passed into the reaction vial at RT until radioactivity reached a maximum (~2 min), and then the reaction vial was isolated and heated at 80 °C for 3 min to produce (*Z*)-*tert*-butyl 4-((2-((1*H*-indazol-3-yl)methylene)-6-[^{11}C]methoxy-3-oxo-2,3-dihydrobenzofuran-7-yl)methyl)piperazine-1-carboxylate ([^{11}C]**3**). Then, a solution of 1 N HCl (500 μL) was introduced to the reaction vial. The reaction mixture was sealed and heated at 80 °C for 3 min. The contents of the reaction vial were diluted with $NaHCO_3$ (0.1 M, 1 mL), and injected onto the semi-preparative HPLC column with 3 mL injection loop for purification. The product fraction was collected in a recovery vial containing 30 mL water. The diluted tracer solution was then passed through a C-18 Sep-Pak Plus cartridge, and washed with water (5 mL × 4). The cartridge was eluted with EtOH (1 mL × 2), followed by 10 mL saline, to release [^{11}C]**4**. The eluted product was then sterile-filtered through a sterile vented Millex-GS 0.22 μm cellulose acetate membrane, and collected into a sterile vial. Total radioactivity (2.3–4.9 GBq) was assayed and total volume (10–11 mL) was noted for tracer dose dispensing. The overall synthesis, purification and formulation time was 30–40 min from EOB. Retention times in the analytical HPLC system were: t_R **5** = 4.27 min, t_R **3** = 5.16 min, t_R **4** = 3.23 min, t_R [^{11}C]**3** = 5.16 min, and t_R [^{11}C]**4** = 3.23 min. Retention times in the semi-preparative HPLC system were: t_R **5** = 4.12 min, t_R **3** = 9.35 min, t_R **4** = 6.78 min, t_R [^{11}C]**3** = 9.35 min, and t_R [^{11}C]**4** = 6.78 min. The radiochemical yields were 20–30% decay corrected to EOB, based on [^{11}C]CO_2.