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The first synthesis of [^{11}C]SB-216763, a new potential PET agent for imaging of glycogen synthase kinase-3 (GSK-3)

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ABSTRACT

SB-216763 is a novel, potent and selective glycogen synthase kinase-3 (GSK-3) inhibitor with an IC_{50} value of 34 nM. [^{11}C]SB-216763 (3-(2,4-dichlorophenyl)-4-(1-[^{11}C]methyl-1*H*-indol-3-yl)-1*H*-pyrrole-2,5-dione), a new potential PET agent for imaging of GSK-3, was first designed and synthesized in 20–30% decay corrected radiochemical yield and 370–555 GBq/μmol specific activity at end of bombardment (EOB). The synthetic strategy was to prepare a carbon-11-labeled maleic anhydride intermediate followed by the conversion to maleimide.

Glycogen synthase kinase-3 (GSK-3) is a serine/threonine protein kinase, which exists as two isoforms (GSK-3α and GSK-3β) with molecular weights of 51 and 46 kDa, respectively.[1–4] GSK-3 is a principal physiological substrate of protein kinase B (PKB, also known as Akt) and the activity of GSK-3 is inhibited by PKB/Akt-mediated phosphorylation in response to certain growth factor stimulation such as nerve growth factor (NGF) and glial cell line-derived neurotrophic factor (GDNF).[1–4] GSK-3 plays an important role in a number of diverse cellular processes including glycogen synthesis in skeletal muscle, neuronal cell survival and alleviation of hyperglycemia via increased glycogen synthesis, even in insulin-resistant cells, and apoptosis.[5,6] GSK-3 expression and activity are associated with various disease processes, and GSK-3 inhibitors have arisen as promising drugs for the treatment of diabetes, neurodegenerative conditions such as Alzheimer's disease, neurological disorders such as bipolar disorder, stroke, inflammation and cancer.[7] SB-216763 (3-(2,4-dichlorophenyl)-4-(1-methyl-1*H*-indol-3-yl)-1*H*-pyrrole-2,5-dione) is a novel, potent and selective GSK-3 inhibitor with an IC_{50} value of 34 nM originally developed by GlaxoSmithKline.[1–6] It is a structurally distinct maleimide, and bisarylmaleimide protein kinase inhibitors have attracted great interest from synthetic and medicinal chemists.[8] Carbon-11-labeled SB-216763 may serve as a new probe for the biomedical imaging technique positron emission tomography (PET), and enable non-invasive monitoring of enzyme GSK-3 in diseases.[8] To radiolabel therapeutic agents as diagnostic agents for imaging of GSK-3 and monitoring of therapeutic efficacy of GSK-3 inhibitors, we have first designed and synthesized [^{11}C]SB-216763 (3-(2,4-dichlorophenyl)-4-(1-[^{11}C]methyl-1*H*-indol-3-yl)-1*H*-pyrrole-2,5-dione) as a new potential PET agent.

There is very limited synthetic information regarding the target compound SB-216763 appearing in the literature. Wishing to study this compound in this laboratory, we decided to make our own material by adopting the available literature methods from our previous work.[8] As illustrated in Scheme 1, preparation of reference standard SB-216763 started with the methylation of commercially available indole with CH_3I in the presence of NaH in DMF to afford *N*-methyl indole (**1**) in 95% yield. Treatment of compound **1** with oxalyl chloride in Et_2O formed *N*-methyl indole-3-glyoxylyl chloride (**2**) in 83% yield, which was used without further purification to couple with 2,4-dicholorphenylacetic acid in the presence of Et_3N in CH_2Cl_2 to provide maleic anhydride **3** in 20% yield. Amination of anhydride **3** to maleimide **4** (SB-216763) was accomplished with hexamethyldisilazane (HMDS) in MeOH/DMF in 80% yield.

Alternate route to achieve SB-216763 was designed using a more simple synthetic protocol developed by Faul et al.[9,10] The procedure involved a one-step condensation of substituted (aryl or indolyl)acetamides with substituted (aryl or indolyl)glyoxyl esters in the presence of KOBu^t in THF. As depicted in Scheme 2, compound **1** was reacted with oxalyl chloride, followed by sodium methoxide to give *N*-methyl indole-3-glyoxalates (**5**) in 34% yield. 2,4-Dichlorophenylacetic acid was first converted into acyl

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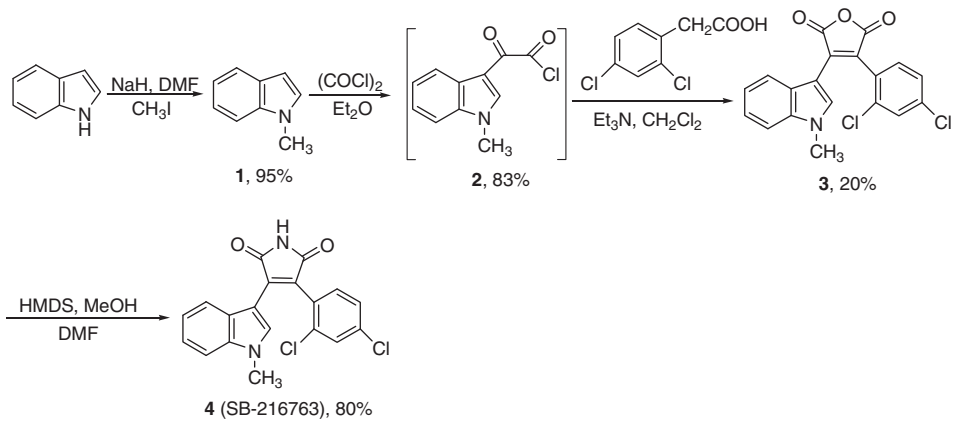
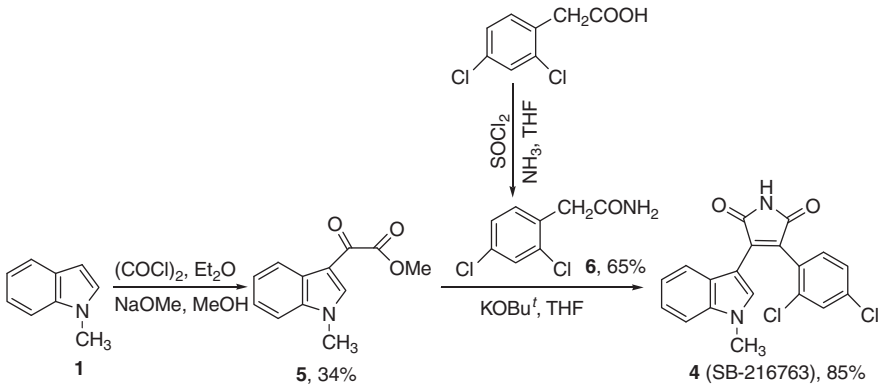


Scheme 1. Synthesis of SB-216763.

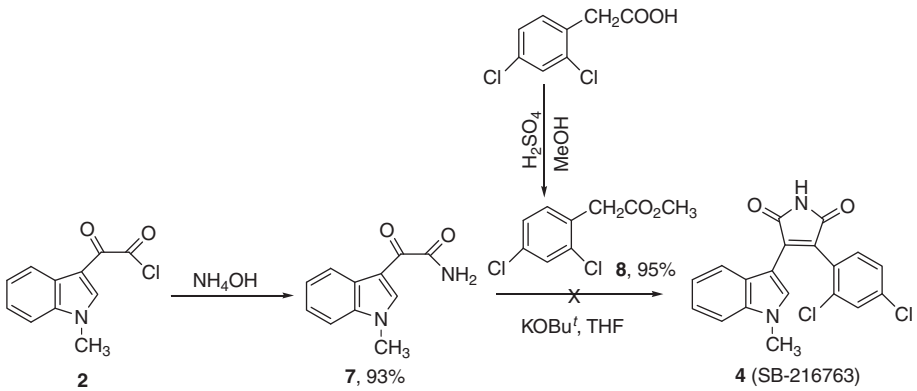


Scheme 2. Alternate synthetic route to SB-216763.

chloride with $SOCl_2$, followed by amination using ammonia in THF to obtain 2-(2,4-dichlorophenyl)acetamide (**6**) in 65% yield. Condensation of glyoxyl ester **5** with acetamide **6** using $KOBu^t$ in THF obtained SB-216763 in 85% yield.

Recently, another convenient method to synthesize indolylaryl and indolylheteroarylmaleimides were reported by the Gribble group.[11] In this method, the maleimides were prepared by condensation of *N*-methyl indole-3-glyoxylamide with aryl acetates in the presence of $KOBu^t$ in THF. Using this methodology, we designed another synthetic approach for SB-216763. As shown in Scheme 3, *N*-methyl indole-3-glyoxylamide (**7**) was prepared by amination of compound **2** with aqueous ammonium hydroxide in 93% yield. Conversion of 2,4-dichlorophenylacetic acid to methyl ester **8** was accomplished with H_2SO_4 in MeOH in 95% yield. However, the condensation reaction of compound **7** with compound **8** failed to provide the expected SB-216763.

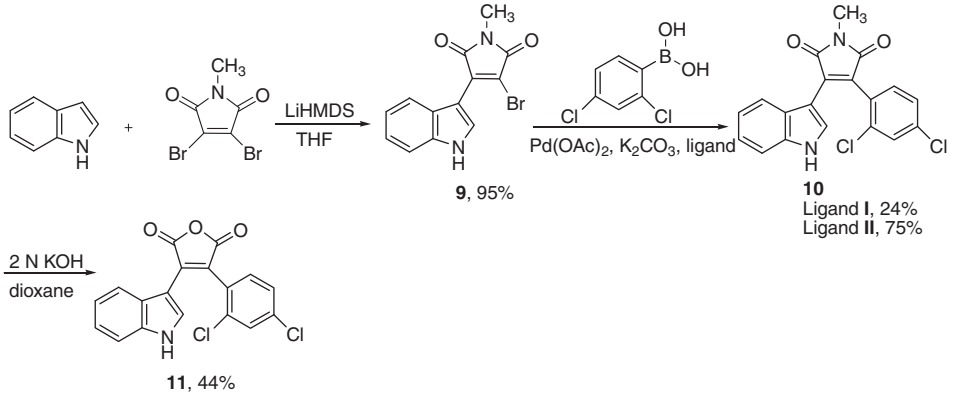
SB-216763 is an attractive molecular target for ^{11}C-labeling. However, the pK_a value of maleimide (about 10.0) is much lower than that of indole (about 21.0),[12] typical ^{11}C-methylation with the most commonly used ^{11}C-methylating reagent, [^{11}C]methyl iodide ([^{11}C]CH_3I) or [^{11}C]methyl triflate ([^{11}C]CH_3OTf),[13,14] will yield mainly on the maleimide nitrogen, rather than on the indole nitrogen. To avoid the competitive ^{11}C-methylation at maleimide nitrogen position, the suitable ^{11}C-labeling precursor was designed



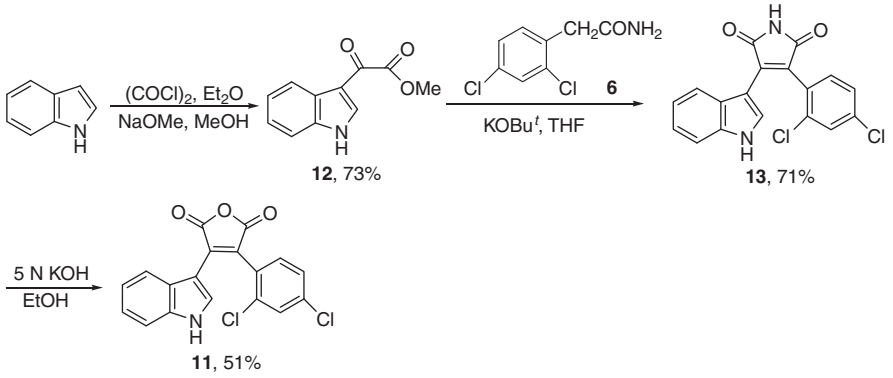
Scheme 3. An unsuccessful synthetic route to SB-216763.

and synthesized. Since indolylaryl maleic anhydride is mild enough to tolerate a wide range of substituent functionalities and can be easily converted to maleimide in the presence of ammonia or an ammonia source, we choose the bisarylmaleic anhydride **11** as the precursor of SB-216763 for ^{11}C-labeling. In our previous work, we have reported the preparation of the precursor of MKC-1 using this synthetic protocol, which involved the reaction of protected 3-indoly acetic acid with indole-3-glyoxylyl chloride to obtain unsymmetrically bisindolyl maleic anhydride.[8] Retrosynthetic analysis suggested that indolylaryl maleic anhydride **11** could be obtained by coupling of 2-(2,4-dichlorophenyl)-2-oxoacetyl chloride with protected 3-indolyl acetic acid in a similar way. However, to the best of our knowledge, 2-(2,4-dichlorophenyl)-2-oxoacetyl chloride is a new compound and difficult to prepare. Therefore, we had to devise another strategy to prepare the precursor. As indicated in Scheme 4, monoindolyl maleimide bromide **9** was obtained in 95% yield from the reaction of commercially available indole and 3,4-dibromo-*N*-methyl maleimide in the presence of hexamethyldisilazane lithium salt (LiHMDS) in THF.[15] Palladium-catalyzed cross-coupling reaction of bromomaleimidoindole **9** with 2,4-dichlorophenyl boronic acid was performed in the presence of 2 mol % $Pd(OAc)_2$ and two different ligands, *n*-butyl-di-1-adamantylphosphine (**I**) and triphenylphosphine (**II**).[16] The catalyst-ligand (**II**) system gave a higher yield (75%) for maleimide **10** compared to that of catalyst-ligand (**I**) system (24%). Hydrolysis of maleimide **10** with 2 N KOH in dioxane afforded the desired precursor **11** in 44% yield. Alternate strategy utilized to prepare indolylaryl maleic anhydride **11** is outlined in Scheme 5. Conversion of indole with oxalyl chloride, followed by sodium methoxide afforded indole-3-glyoxalates **12** in 73% yield, which was condensed with compound **6** in the presence of KOBu^t in THF to obtain maleimide **13** in 71% yield. Hydrolysis of compound **13** with 5 N KOH in EtOH yielded the precursor **11** in 51% yield.

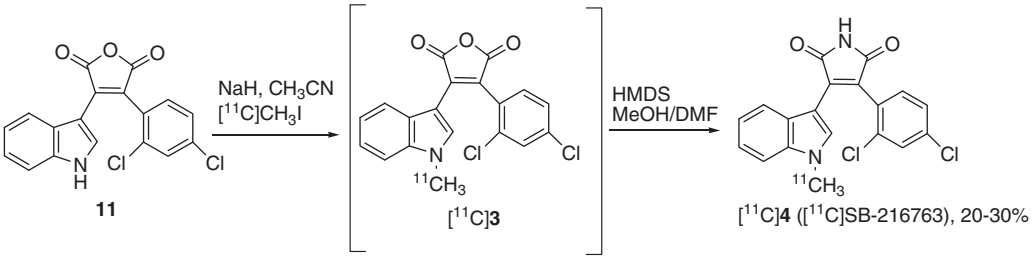
Synthesis of the target radiotracer [^{11}C]SB-216763 ([^{11}C]**4**) is indicated in Scheme 6. Precursor **11** was labeled by [^{11}C]CH_3I prepared from [^{11}C]CO_2,[13,14] in the presence of NaH in acetonitrile through the N-[^{11}C]methylation[8] to provide a radiolabeling intermediate [^{11}C]**3**. Without further purification, [^{11}C]**3** was quickly converted to the target tracer [^{11}C]**4** using HMDS in MeOH/DMF. [^{11}C]**4** was isolated from the radiolabeling mixture by the semi-preparative reversed-phase high performance liquid chromatography (HPLC) in 20–30% radiochemical yields, decay corrected to end of bombardment (EOB), based on [^{11}C]CO_2. Another more reactive radiolabeled precursor [^{11}C]CH_3OTf[13,14] was also used in the radiolabeling reaction of the precursor **11**, but the radiochemical yield was much lower than that of [^{11}C]CH_3I. This suggested it is better to use [^{11}C]CH_3I for the N-[^{11}C]methylation of indole nitrogen. The synthesis was performed in an automated multi-purpose ^{11}C-radiosynthesis module, allowing measurement of specific activity during synthesis.[17,18] The specific activity of [^{11}C]**4** was in a range of 370–555 GBq/μmol at EOB measured by the on-the-fly technique using semi-preparative HPLC during synthesis[18] and 185–278 GBq/μmol at the end of synthesis (EOS) determined by analytical HPLC,[18] respectively. Chemical purity and radiochemical purity were determined by analytical HPLC.[19] The chemical purity of the precursor **11**, 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 >93% determined by reversed-phase HPLC through UV flow detector.



Scheme 4. Synthesis of maleic anhydride precursor.



Scheme 5. Alternate synthetic route to maleic anhydride precursor.



Scheme 6. Synthesis of [^{11}C]SB-216763.

The synthetic information of SB-216763 was limited in the literature. Thus, the experimental details and characterization data for compounds **1–13** and for the tracer [^{11}C]**4** are given.[20]

In summary, [^{11}C]SB-216763 was first designed and synthesized as a new potential PET agent for imaging of GSK-3 in various diseases. An automated multi-purpose ^{11}C-radiosynthesis module of our own design for fully automated synthesis of [^{11}C]SB-216763 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 N-[^{11}C]methylation radiolabeling on indole nitrogen of the precursor incorporated efficiently with the most commonly used [^{11}C]methylating agent, [^{11}C]CH_3I, produced through a signature reaction of carbon-11 radiochemistry 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 [^{11}C]SB-216763 in animals and humans.

Acknowledgments

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20. (a) General. All commercial reagents and solvents from Sigma–Aldrich and Fisher Scientific were used without further purification. [^{11}C]CH_3Br was prepared according to a literature procedure,[14] and [^{11}C]CH_3I was prepared from [^{11}C]CH_3Br by passing through a NaI column (NaI mixed with Carbograph™-2 60/80, Grace, 1:1 w/w) at 300 °C. Melting points were determined on a MEL-TEMP II capillary tube apparatus and were uncorrected. ^{1}H NMR spectra were recorded on Bruker Avance II 500 MHz NMR spectrometers 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). Liquid chromatography–mass spectra (LC–MS) analysis was performed on an Agilent system, consisting of a 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 Thermo MAT 95XP-Trap 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. Preparative TLC was run using Analtech silica gel UV 254 plates (20 × 20 cm^2). 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/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; 3:1:1 CH_3CN/MeOH/20 mM, pH 6.7 phosphate (buffer solution) mobile phase; flow rate 1.5 mL/min; and UV (254 nm) and γ-ray (PIN diode) flow detectors. Semi-preparative HPLC was performed using a YMC-Pack ODS-A, S-5 μm, 12 nm, 10 × 250 mm C-18 column; 3:1:1 CH_3CN/MeOH/20 mM, pH 6.7 phosphate (buffer solution) mobile phase; 5.0 mL/min flow rate; UV (254 nm) and γ-ray (PIN diode) flow detectors. Sterile Millex-GS 0.22 μm vented filter unit was obtained from Millipore Corporation, Bedford, MA. (b) 1-Methyl-1*H*-indole (**1**). To a stirred solution of indole (10.0 g, 85.0 mmol) in DMF (200 mL) was added NaH (60% dispersion in mineral oil, 5.12 g, 128.0 mmol) in small portions to at 0 °C. After stirring at ambient temperature for 1 h, the reaction mixture was cooled to 0 °C. Methyl iodide (8.3 mL, 133.0 mmol) was added dropwise, and the reaction mixture was slowly allowed to warm to ambient temperature and stirred overnight. The mixture was then poured into ice-water and extracted with EtOAc. The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was removed in vacuo, and the crude product was purified by column chromatography (4:1 hexanes/EtOAc) to give **1** (10.65 g, 95%) as a clear oil: ^{1}H NMR ($CDCl_3$)δ 7.63 (td, J = 1.0, 8.0 Hz, 1H), 7.32 (d, J = 8.0 Hz, 1H), 7.24–7.20 (m, 1H), 7.12–7.09 (m, 1H), 7.04 (d, J = 7.0 Hz, 1H), 6.48 (d, J = 7.5 Hz, 1H), 3.78 (s, 3H). (c) 2-(1-Methyl-1*H*-indol-3-yl)-2-oxoacetyl chloride (**2**). To a stirred solution of compound **1** (6.0 g, 46.0 mmol) in Et_2O (100 mL) was added oxalyl chloride (8.0 mL, 92.0 mmol) dropwise at 0 °C. After stirring for 3 h at same temperature, the precipitate was collected by filtration, rinsed with cooled Et_2O and dried to give **2** (8.37 g, 83%) as a yellow solid. This compound was used without further purification. (d) 3-(2,4-Dichlorophenyl)-4-(1-methyl-1*H*-indol-3-yl)furan-2,5-dione (**3**). To a stirred solution of 2,4-dicholorphenylacetic acid (2.0 g, 9.8 mmol) and triethylamine (2.73 mL, 19.6 mmol) in CH_2Cl_2 (100 mL) was added compound **2** (2.15 g, 9.8 mmol) in small portions. After stirring overnight at ambient temperature, the solvent was removed in vacuo. The residue was separated using column chromatography with (100:1 CH_2Cl_2/MeOH) to provide the red crude product, which was further purified by column chromatography with (2:1 hexanes/acetone) to give **3** (730.0 mg, 20%) as a red solid: mp 188–190 °C; ^{1}H NMR (DMSO-d_6) δ 8.31 (s, 1H), 7.81 (d, J = 2.5 Hz, 1H), 7.57–7.54 (m, 2H), 7.50 (d, J = 8.5 Hz, 1H), 7.21 (td, J = 1.0, 8.0 Hz, 1H), 6.85 (td, J = 0.5, 8.0 Hz, 1H), 6.30 (d, J = 8.0 Hz, 1H), 3.93 (s, 3H); HRMS (CI, m/z): calcd for $C_{19}H_{12}NO_3Cl_2$ ([M+H]$^+$) 372.0189, found 372.0189. (e) 3-(2,4-Dichlorophenyl)-4-(1-methyl-1*H*-indol-3-yl)-1*H*-pyrrole-2,5-dione (SB-216763, **4**). To a stirred solution of compound **3** (250 mg, 0.7 mmol) in DMF (5 mL) was added HMDS (1.4 mL, 6.7 mmol), followed by MeOH (140 μL, 3.4 mmol). After stirring at ambient temperature

overnight, the reaction mixture was poured into water and extracted with EtOAc. The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was removed in vacuo, and the crude product was purified by preparative TLC plate (100:3 CH_2Cl_2/MeOH) to give **4** (200.0 mg, 80%) as a red solid: mp 102–104 °C; ^{1}H NMR (DMSO-d_6) δ 11.19 (s, 1H), 8.11 (s, 1H), 7.72 (d, J = 2.0 Hz, 1H), 7.50–7.47 (m, 2H), 7.41 (d, J = 8.5 Hz, 1H), 7.14 (td, J = 1.0, 8.0 Hz, 1H), 6.79 (td, J = 1.0, 8.0 Hz, 1H), 6.35 (d, J = 8.0 Hz, 1H), 3.89 (s, 3H); HRMS (EI, m/z): calcd for $C_{19}H_{12}N_2O_2Cl_2$ ([M]$^+$) 370.0270, found 370.0268. (f) Methyl 2-(1-methyl-1*H*-indol-3-yl)-2-oxoacetate (**5**). To a solution of compound **1** (1.0 g, 7.6 mmol) in Et_2O (10 mL) was added oxalyl chloride (0.7 mL, 8.02 mmol) dropwise at 0 °C. The yellow slurry was stirred at same temperature for 0.5 h and then cooled to −78 °C. A solution of sodium methoxide in MeOH (25 wt %, 4.5 mL, 19.7 mmol) was added to this slurry at same temperature. After addition, the reaction mixture was allowed to warm to room temperature and quenched with water (5 mL). The precipitate was collected by filtration, rinsed with water, Et_2O and dried to give **5** (565 mg, 34%) as a tan solid: mp 80–81 °C; ^{1}H NMR (DMSO-d_6) δ 8.50 (s, 1H), 8.18 (d, J = 7.5 Hz, 1H), 7.62 (d, J = 8.0 Hz, 1H), 7.38–7.31 (m, 2H), 3.92 (s, 3H), 3.90 (s, 3H). (g). 2-(2,4-Dichlorophenyl)acetamide (**6**). A suspension of 2,4-dichlorophenylacetic acid (2.0 g, 9.8 mmol) in thionyl chloride (7 mL) was stirred at ambient temperature overnight. The excess thionyl chloride was removed in vacuo, keeping the temperature below 30 °C. The residue was taken up into toluene and the solvent was removed in vacuo. This operation is repeated three times to remove the thionyl chloride. The residue was re-dissolved in THF (50 mL), and ammonia gas is bubbled through the solution, keeping the temperature below 10 °C. The resulting suspension was diluted with cold water to get a clear solution, which was concentrated to small volume. The precipitate was collected by filtration, rinsed with 1:8 THF/H_2O and dried to give **6** (1.73 g, 65%) as a white solid: mp 168–169 °C (lit.[21] 168–169 °C); ^{1}H NMR (DMSO-d_6) δ 7.56 (s, 1H), 7.49 (br s, 1H), 7.40–7.36 (m, 2H), 7.00 (br s, 1H), 3.55 (s, 2H). (h). Alternate synthetic procedure for compound **4**. To a stirred suspension of compound **5** (250.0 mg, 1.2 mmol) and compound **6** (117.3 mg, 0.58 mmol) in THF (10 mL) was added 1.0 M KOBu^t in THF (2.6 mL, 2.6 mmol) under nitrogen atmosphere. After stirring at ambient temperature for 4 h, the reaction mixture was quenched with 1 N HCl (8 mL) and extracted with EtOAc. The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was removed in vacuo, and the crude product was purified by column chromatography with (2:1 hexanes/acetone) to give **4** (182.0 mg, 85%) as a red solid. The analytical data were obtained as same as above. (i) 2-(1-Methyl-1*H*-indol-3-yl)-2-oxoacetamide (**7**). To a concentrated ammonium hydroxide solution (40 mL) was added compound **2** (1.5 g, 6.8 mmol) in small portions at 0 °C. The reaction mixture was stirred at same temperature for 1 h. The precipitate was collected by filtration, rinsed with water, Et_2O and dried to give **7** (1.28 g, 93%) as a pale yellow solid: mp 186–188 °C (lit.[11] 186–188 °C); ^{1}H NMR (DMSO-d_6) δ 8.74 (s, 1H), 8.26–8.24 (m, 1H), 8.08 (br s, 1H), 7.73 (br s, 1H), 7.60–7.58 (m, 1H), 7.35–7.30 (m, 2H), 3.92 (s, 3H). (j) Methyl 2-(2,4-dichlorophenyl)acetate (**8**). To a solution of 2,4-dichlorophenylacetic acid (3.5 g, 17.1 mmol) in MeOH (100 mL) was added concentrated H_2SO_4 (20 drops). After the reaction mixture was heated at reflux overnight, the solvent was removed in vacuo. The residue was diluted with cold water and extracted with EtOAc. The combined organic layer was washed with saturated $NaHCO_3$, brine, dried over Na_2SO_4, and filtered. The solvent was removed in vacuo, and the crude product was purified by column chromatography with (4:1 hexanes/EtOAc) to give **8** (3.56 g, 95%) as a clear oil: ^{1}H NMR (DMSO-d_6) δ 7.40 (s, 1H), 7.22 (d, J = 1.5 Hz, 2H), 3.74 (s, 2H), 3.71 (s, 3H). (k) 3-Bromo-4-(1*H*-indol-3-yl)-1-methyl-1*H*-pyrrole-2,5-dione (**9**). To a stirred solution of indole (2.47 g, 21.1 mmol) in THF (30 mL) was added a solution of LiHMDS (1.0 M in THF, 50 mL, 50.0 mmol) dropwise under nitrogen atmosphere at −20 °C. After stirring for 45 min at same temperature, a solution of 2,3-dibromo-*N*-methylmaleimide (5.0 g, 18.6 mmol) in THF (30 mL) was added dropwise at −20 °C, followed by stirring for 30 min at 0 °C. The reaction mixture was poured into cooled 0.2 N HCl and extracted with EtOAc. The combined organic layer was washed with saturated $NaHCO_3$, brine, dried over Na_2SO_4, and filtered. The solvent was removed in vacuo, and the residue was recrystallized from MeOH to give **9** (5.4 g, 95%) as a red solid: mp 144 °C (dec.) (lit.[22,23] 145 °C (dec.)); ^{1}H NMR (DMSO-d_6) δ 12.12 (s, 1H), 8.06 (d, J = 3.0 Hz, 1H), 7.91 (d, J = 8.0 Hz, 1H), 7.51 (d, J = 8.0 Hz, 1H), 7.23 (td, J = 1.0, 8.0 Hz, 1H), 7.15 (td, J = 1.0, 8.0 Hz, 1H), 3.01 (s, 3H). (l) 3-(2,4-Dichlorophenyl)-4-(1*H*-indol-3-yl)-1-methyl-1*H*-pyrrole-2,5-dione (**10**) using *n*-butyl-di-1-adamantylphosphine as ligand (**I**). In an Ace-pressure tube, 1 M K_2CO_3 (1.5 mL), $Pd(OAc)_2$ (2 mol %) and *n*-butyl-di-1-adamantylphosphine (2 mol %) was added to a stirred solution of compound **9** (152.0 mg, 0.5 mmol) and 2,4-dichlorophenyl boronic acid (143.0 mg, 0.75 mmol) in dimethoxyethane (1.5 mL) under nitrogen atmosphere. The pressure tube was fitted with a Teflon cap and heated at 100 °C overnight. The mixture was cooled to ambient temperature and diluted with EtOAc. The organic layer was washed with saturated NH_4Cl, brine, dried over Na_2SO_4, and filtered. The solvent was removed in vacuo, and the crude product was purified by preparative TLC plate column chromatography (2:1 hexanes/acetone) to give **10** (45.0 mg, 24%) as a red solid: 200 °C (dec.); ^{1}H NMR ($CDCl_3$) δ 8.80 (br s, 1H), 8.08 (d, J = 3.0 Hz, 1H), 7.44 (d, J = 2.0 Hz, 1H), 7.36 (d, J = 8.0 Hz, 1H), 7.31–7.26 (m, 2H), 7.15 (t, J = 7.5 Hz, 1H), 6.85 (t, J = 8.0 Hz, 1H), 6.49 (d, J = 8.5 Hz, 1H), 3.18 (s, 3H); MS (ESI, m/z): 371 ([M+H]$^+$, 100%); HRMS (EI, m/z): calcd for $C_{19}H_{12}N_2O_2Cl_2$ ([M]$^+$) 370.0270, found 370.0263. Compound **10** using triphenylphosphine as ligand (**II**). In an Ace-pressure tube, 1 M K_2CO_3 (3 mL), $Pd(OAc)_2$ (2 mol %) and triphenylphosphine (2 mol %) was added to a stirred solution of compound **9** (304.0 mg, 1.0 mmol) and 2,4-dichlorophenyl boronic acid (286.0 mg, 1.5 mmol) in dimethoxyethane (3 mL) under nitrogen atmosphere. The pressure tube was fitted with a Teflon cap and heated at 100 °C overnight. The mixture was cooled to ambient temperature and diluted with EtOAc. The organic layer was washed with saturated NH_4Cl, brine, dried over Na_2SO_4, and filtered. The solvent was removed in vacuo, and the crude product was purified by preparative TLC plate column chromatography (2:1 hexanes/acetone) to give **10** (280.0 mg, 75%) as a red solid: MS (ESI, m/z): 371 ([M+H]$^+$, 100%). (m) 3-(2,4-Dichlorophenyl)-4-(1*H*-indol-3-yl)furan-2,5-dione (**11**). To a solution of compound **10** (120.0 mg, 0.32 mmol) in dioxane (8 mL) was added 2 N KOH (10 mL). After the reaction mixture was heated at reflux overnight, it was poured into ice-water and acidified to pH 2.0 with 5 N HCl. The mixture was then extracted with EtOAc. The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was removed in vacuo, and the crude product was purified by preparative TLC plate (2:1 hexanes/acetone) to give **11** (51.0 mg, 44%) as a red solid: mp 220–221 °C; ^{1}H NMR (DMSO-d_6) δ 12.35 (s, 1H), 8.22 (s, 1H), 7.82 (d, J = 2.0 Hz, 1H), 7.58 (dd, J = 2.0, 8.5 Hz, 1H), 7.51 (d, J = 8.0 Hz, 1H), 7.48 (d, J = 8.0 Hz, 1H), 7.13 (td, J = 1.0, 8.0 Hz, 1H), 6.81 (td, J = 1.0, 8.0 Hz, 1H), 6.34 (d, J = 8.0 Hz, 1H); MS (ESI, m/z): 358 ([M+H]$^+$, 100%); HRMS (EI, m/z): calcd for $C_{18}H_9NO_3Cl_2$ ([M]$^+$) 356.9954, found 356.9967. (n) Methyl 2-(1*H*-indol-3-yl)-2-oxoacetate (**12**). To a solution of indole (2.0 g, 17.1 mmol) in Et_2O (20 mL) was added oxalyl chloride (1.5 mL, 17.2 mmol) dropwise at 0 °C. The yellow slurry was stirred at same temperature for 0.5 h and then cooled to −78 °C. A solution of NaOMe in MeOH (25 wt %, 7.8 mL, 34.1 mmol) was added to this slurry at same temperature. After addition, the reaction mixture was allowed to warm to ambient temperature, and quenched by addition of H_2O (10 mL). The precipitate was collected by filtration, washed with H_2O and dried to give **12** (2.5 g, 73%) as a yellow solid: mp 160 °C (dec.); ^{1}H NMR (DMSO-d_6) δ 12.42 (s, 1H), 8.45 (d, J = 3.5 Hz, 1H), 8.16 (dd, J = 1.5, 6.0 Hz, 1H), 7.55 (dd, J = 1.5, 6.0 Hz, 1H), 7.32–7.27 (m, 2H), 3.89 (s, 3H). (o) 3-(2,4-Dichlorophenyl)-4-(1*H*-indol-3-yl)-1*H*-pyrrole-2,5-dione (**13**). To a suspension of compound **12** (1.17 g, 5.7 mmol) and compound **6** (586.5 mg, 2.9 mmol) in THF (50 mL) was added 1.0 M KOBu^t in THF (11.5 mL, 11.5 mmol) under nitrogen atmosphere. After stirring at ambient temperature for 5 h, the reaction mixture was quenched with 1 N HCl (50 mL) and extracted with EtOAc. The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was removed in vacuo, and the crude product was purified by column chromatography with (2:1 hexanes/acetone) to give **13** (726.0 mg, 71%) as an orange solid: mp 254–256 °C; ^{1}H NMR (DMSO-d_6) δ 12.02 (s, 1H), 11.19 (s, 1H), 8.04 (d, J = 3.0 Hz, 1H), 7.73 (d, J = 2.0 Hz, 1H), 7.49 (dd, J = 2.0, 8.5 Hz, 1H), 7.43 (dd, J = 3.0, 8.5 Hz, 2H), 7.09–7.06 (m, 1H), 6.76–6.73 (m, 1H), 6.40(d, J = 8.0 Hz, 1H); HRMS (EI, m/z): calcd for $C_{18}H_{10}N_2O_2Cl_2$ ([M]$^+$) 356.0114, found 356.0126. (p) Alternate synthetic procedure for compound **11**. To a suspension of compound **13** (200.0 mg, 0.56 mmol) in EtOH (3 mL) was added 5 N KOH (8 mL). After the reaction mixture was heated at reflux overnight, EtOH was removed in vacuo. The residue was acidified to pH 2.0 with 5 N HCl. The mixture was then extracted with EtOAc. The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was removed in vacuo, and the crude product was purified by preparative TLC plate (2:1 hexanes/acetone) to give **11** (103.0 mg, 51%) as a red solid: MS (ESI, m/z): 358 ([M+H]$^+$, 100%). (q) 3-(2,4-Dichlorophenyl)-4-(1-[^{11}C]methyl-1*H*-indol-3-yl)-1*H*-pyrrole-2,5-dione ([^{11}C]SB-216763, [^{11}C]**4**). [^{11}C]CO_2 was produced by the ^{14}N(p,α)^{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 proton-beam current was 55 μA, and the irradiation time was 30 min. The precursor **11** (0.1–0.3 mg) was dissolved in CH_3CN (300 μL). To this solution was added NaH (1 mg). The mixture was transferred to a small reaction vial. No-carrier-added (high specific activity) [^{11}C]CH_3I that was produced by the gas-phase production method[14] from [^{11}C]CO_2 through [^{11}C]CH_4 and [^{11}C]CH_3Br with NaI column was passed into the reaction vial at 0 °C until radioactivity reached a maximum (~2 min), and then the reaction vial was isolated and heated at 45 °C for 4 min to produce 3-(2,4-dichlorophenyl)-4-(1-[^{11}C]methyl-1*H*-indol-3-yl)furan-2,5-dione ([^{11}C]**3**). Then, a solution of HMDS (2 μL) and MeOH (2 μL) in DMF (300 μL) was introduced to the reaction vial. The reaction mixture was sealed and heated at 80 °C for 8 min. The contents of the reaction vial were diluted with $NaHCO_3$ (1 mL, 0.1 M), and injected onto the semi-preparative HPLC column with 2 mL injection loop. The product fraction was collected, the solvent was removed by rotatory evaporation under vacuum, and the final product, [^{11}C]**4**, was formulated in saline, 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 for tracer dose dispensing. The overall synthesis, purification and formulation time was 35–40 min from EOB. Retention times in the analytical HPLC system were: t_R **11** = 5.61 min, t_R **3** = 7.48 min, t_R **4** = 5.11 min, t_R [^{11}C]**3** = 7.48 min, and t_R [^{11}C]**4** = 5.11 min. Retention times in the semi-preparative HPLC system were: t_R **11** = 7.23 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.

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