Synthesis of [^{11}C]CX-6258 as a new PET tracer for imaging of Pim kinases in cancer

Min Wang[a], Reynaldo Tzintzun[a], Mingzhang Gao[a], Zhidong Xu[b], Qi-Huang Zheng[a,*]

[a]*Department of Radiology and Imaging Sciences, Indiana University School of Medicine, 1345 West 16th Street, Room 202, Indianapolis, IN 46202, USA*

[b]*Key Laboratory of Medicinal Chemistry and Molecular Diagnosis of Ministry of Education, College of Chemistry and Environmental Science, Hebei University, Baoding, Hebei 071002, China*

*Corresponding author. Tel.: +1 317-278-4671. Fax: +1 317-278-9711. E-mail address: qzheng@iupui.edu.

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Abstract—The reference standard CX-6258 {(*E*)-5-chloro-3-((5-(3-(4-methyl-1,4-diazepane-1-carbonyl)phenyl)furan-2-yl)methylene)indolin-2-one, **4a**} and its desmethylated precursor *N*-desmethyl-CX-6258 {(*E*)-3-((5-(3-(1,4-diazepane-1-carbonyl)phenyl)furan-2-yl)methylene)-5-chloroindolin-2-one, **5**} for radiolabeling were synthesized from 5-bromo-2-furaldehyde and 3-carboxybenzeneboronic acid in 3 and 4 steps with 29-49% and 24-32% overall chemical yield, respectively. The target tracer [^{11}C]CX-6258 {(*E*)-5-chloro-3-((5-(3-(4-[^{11}C]methyl-1,4-diazepane-1-carbonyl)phenyl)furan-2-yl)methylene)indolin-2-one, [^{11}C]**4a**} was prepared from *N*-desmethyl-CX-6258 (**5**) with [^{11}C]CH_3OTf under basic condition (2 N NaOH) through *N*-[^{11}C]methylation and isolated by HPLC combined with solid-phase extraction (SPE) in 40-50% radiochemical yield based on [^{11}C]CO_2 and decay corrected to end of bombardment (EOB) with 370-1110 GBq/μmol specific activity at EOB.

Keywords: [^{11}C]CX-6258; Radiosynthesis; Positron emission tomography (PET); Pim kinases; Cancer.

Pim kinases (Provirus Integration site for Moloney murine leukemia virus) are a family of serine/threonine kinases, including three different members Pim1, Pim2 and Pim3.[1-3] This family of kinases regulates signaling pathways via Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway and plays pivotal roles in cancer development and progression.[4-6] Pim kinases including Pim1, Pim2 and Pim3 are overexpressed not only in hematologic malignancies but also in solid tumors, and have become an attractive targets for therapeutics in cancer.[7-10] Many Pim kinases inhibitors have been discovered.[11,12] Recently a potent, selective, and orally efficacious pan-Pim kinases inhibitor called CX-6258 has been developed by Cylene Pharmaceuticals, and the IC_{50} of CX-6258 is 5, 25, and 16 nM for Pim1, Pim2, and Pim3, respectively.[13] Pim kinases have also become a promising target for molecular imaging of Pim kinases-related cancers and image-guided therapy using the biomedical imaging technique positron emission tomography (PET). In our previous work, we have developed a series of radiolabeled (carbon-11 and fluorine-18) protein kinase (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]GSK2126458 and 4-[^{18}F]GSK2126458, as shown in Figure 1, as new cancer imaging agents for PET to study PK and PK inhibitors.[14-20] In this ongoing study, we target Pim kinases. Here we report the design, synthesis and labeling of [^{11}C]CX-6258 (Figure 1).

The reference standard CX-6258 {(*E*)-5-chloro-3-((5-(3-(4-methyl-1,4-diazepane-1-carbonyl)phenyl)furan-2-yl)methylene)indolin-2-one, **4a**} and its [^{11}C]-labeling precursor *N*-desmethyl-CX-6258 {(*E*)-3-((5-(3-(1,4-diazepane-1-carbonyl)phenyl)furan-2-yl)methylene)-5-

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chloroindolin-2-one, **5**} were prepared based on the reported method[13] with modifications. As shown in Scheme 1, Suzuki cross-coupling of 3-carboxybenzeneboronic acid with 5-bromo-2-furaldehyde in the presence of aqueous 2 M Na_2CO_3 using $Pd(OAc)_2$ as catalyst in toluene/EtOH to give 3-(5-formylfuran-2-yl)benzoic acid (**1**) in 89% yield.[21] Compounds **2a** and **2b** were achieved via amide bond coupling of acid **1** with homopiperazines in the presence of *N*,*N*-diisopropylethylamine (DIPEA) using *O*-(benzotriazo-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU) as coupling agent in *N*,*N*-dimethylformamide (DMF) with 75% and 64% yield, respectively.[22] Condensation of 5-chlorooxindole with aldehydes **2a** and **2b** using piperidine as catalyst in EtOH afforded standard compound **4a** and *N*-Boc protected precursor **4b** with 44% and 64% yield, respectively.[23] Compounds **4a** and **4b** could be obtained by reverse the above two steps in much higher yields. Condensation of compound **1** with 5-chlorooxindole using piperidine as catalyst in EtOH to provide acid **3** in 70% yield, which was coupled with homopiperazines using 1,1'-carbonyldimidazole (CDI) as coupling agent in DMF to provide **4a** and **4b** in 78% and 79% yield, respectively. The Boc group of **4b** was removed with trifluoroacetic acid (TFA) in CH_2Cl_2 to afford the precursor **5** in 66% yield.

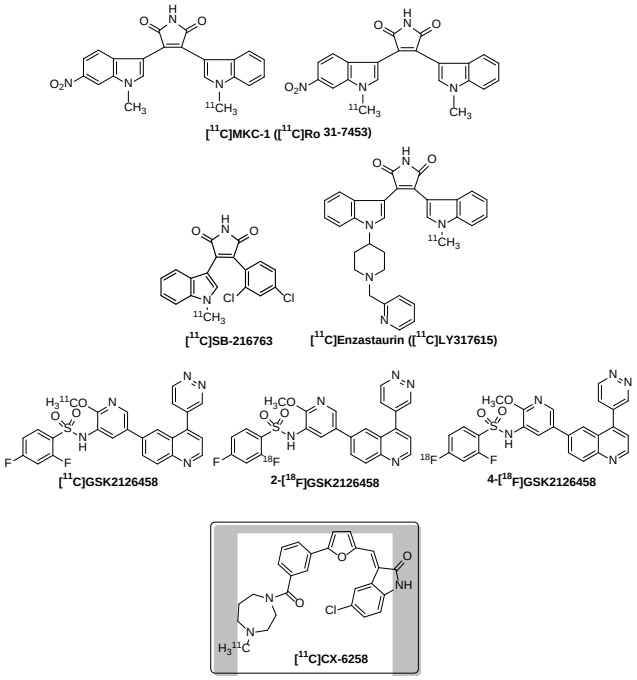
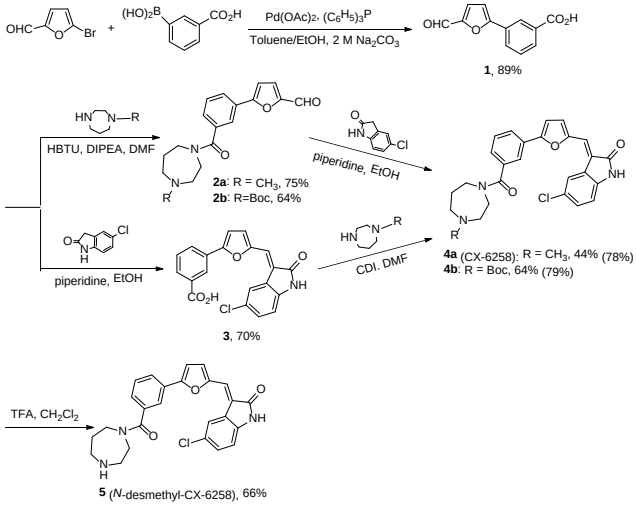
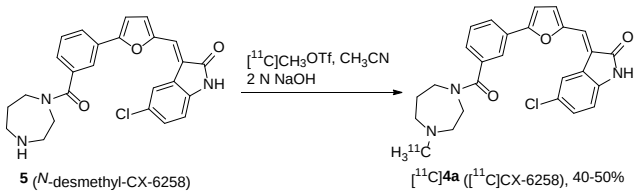


Figure 1. Radiolabeled PK inhibitors.

Synthesis of the target tracer [^{11}C]CX-6258 {(*E*)-5-chloro-3-((5-(3-(4-[^{11}C]methyl-1,4-diazepane-1-carbonyl)phenyl)furan-2-yl)methylene)indolin-2-one, [^{11}C]**4a**} is indicated in Scheme 2. The desmethylated precursor **5** was labeled by [^{11}C]methyl triflate ([^{11}C]CH_3OTf)[24,25] through *N*-[^{11}C]methylation[26-28] at 80 °C under basic condition (2 N NaOH) and isolated by a semi-preparative reverse-phase (RP) high performance liquid chromatography (HPLC) with a C-18 column and a solid-phase extraction (SPE) with a disposable C-18 Plus Sep-Pak cartridge (a second purification or isolation process)[29-31] to produce the corresponding pure radiolabeled compound [^{11}C]**4a** in 40-50% radiochemical yield, decay corrected to end of bombardment (EOB), based on [^{11}C]CO_2. There are two nitrogen positions in the precursor **5** where the *N*-[^{11}C]methylation reaction could potentially happen. However, the reactivity for *N*-[^{11}C]methylation is different between these two positions. The acidity of amine –NH is greater than amide –NH, thus the deprotonization at –NH of amine is easier than at –NH of amide under basic condition, the *N*-[^{11}C]methylation reaction would prefer to occur at amine –NH, and the target tracer [^{11}C]**4a** would be a major product.



Scheme 1. Synthesis of the reference standard CX-6258 (**4a**) and its precursor *N*-desmethyl-CX-6258 (**5**).



Scheme 2. Synthesis of the target tracer [^{11}C]CX-6258.

[^{11}C]CH_3OTf was used as the [^{11}C]-radiolabeled precursor, which is more reactive than another commonly used [^{11}C]-methylation reagent [^{11}C]methyl iodide ([^{11}C]CH_3I)[32], and thus, the radiochemical yield of [^{11}C]**4a** was relatively higher. Basic condition would help the *N*-[^{11}C]methylation of precursor and significantly increase the radiochemical yield of [^{11}C]**4a**. Small amount of the precursor (0.3-0.5 mg) was used for radiolabeling instead of commonly used large amount of the precursor (1.0-1.5 mg), which

improved the chemical purity of the final tracer solution. However, the small amount of precursor could potentially decrease the radiolabeling yield and increase the specific activity of the target tracer by decreasing the radiolabeling product as well as product mass. In addition, in order to make more product radioactivity, we also optimized the semi-preparative HPLC conditions including column, mobile phase and flow rate to shorten the retention time of [^{11}C]**4a** to 8-9 min. Addition of $NaHCO_3$ solution 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]**4a** from its desmethylated precursor **5**.[29-31,33]

The radiosynthesis was performed in a home-built automated multi-purpose [^{11}C]-radiosynthesis module, allowing measurement of specific radioactivity during synthesis.[34-36] This [^{11}C]-radiosynthesis module includes the overall design of the reaction, purification and reformulation capabilities of the prototype system. In addition, [^{11}C]-tracer specific activity (SA, GBq/μmol at EOB) can be automatically determined prior to product delivery for compounds purified by the HPLC-portion of the system.[36,37] The SA was in a range of 370-1110 GBq/μmol at EOB. The 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]CH_3OTf system, [^{11}C]-radiolabeled precursor or called [^{11}C]-radiolabeled methylating reagent. If we can eliminate ^{12}C carrier-added as much as possible, then we will be able to achieve higher SA. The [^{11}C]-gas target we used is the Siemens RDS-111 Eclipse cyclotron [^{11}C]-gas target. The technical trick to produce high SA [^{11}C]CO_2 is we will usually do 2-3 times pre-burn with the same beam current and short time like 10 min before production run. This pre-burn will warm up the cyclotron and eliminate significant amount of ^{12}C carrier-added in the 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.[25]

Chemical purity and radiochemical purity were determined by analytical HPLC.[38] The chemical purity of the precursor **5** and reference standard **4a** was >93%. The radiochemical purity of the target tracer [^{11}C]**4a** was >99% determined by radio-HPLC through γ-ray (PIN diode) flow detector, and the chemical purity of [^{11}C]**4a** was >90% determined by RP HPLC through UV flow detector. A C-18 Plus Sep-Pak cartridge instead of rotatory evaporation was used for purification and isolation process to significantly improve the chemical purity of the tracer solution.[29-31,38] In this study, the Sep-Pak purification further increased the chemical purity >10%.[29-31]

The experimental details and characterization data for compounds **1-5** and for the tracer [^{11}C]**4a** are given.[39]

In summary, a multi-step synthetic route with moderate to high yields for the synthesis of the precursor *N*-desmethyl-CX-6258, reference standard CX-6258 and target tracer [^{11}C]CX-6258 has been developed. An automated self-designed multi-purpose [^{11}C]-radiosynthesis module for the synthesis of [^{11}C]CX-6258 has been built, featuring the measurement of specific activity by the on-the-fly technique. The radiosynthesis employed *N*-[^{11}C]methylation radiolabeling on amine nitrogen position of the precursor. Radiolabeling procedures 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 RP HPLC combined with SPE procedure in high radiochemical yield, radiochemical purity and chemical purity, short overall synthesis time, and high specific activity. These chemistry results warrant further biological evaluation of [^{11}C]CX-6258 as a new PET agent for imaging of Pim kinases in cancer.

Acknowledgments

^{1}H NMR spectra were recorded on a Bruker Avance II 500 MHz NMR spectrometer in the Department of Chemistry and Chemical Biology at Indiana University Purdue University Indianapolis (IUPUI), which is supported by the United States National Science Foundation (NSF) Major Research Instrumentation Program (MRI) grant CHE-0619254.

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39. *(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.[25] Melting points were determined on a MEL-TEMP II capillary tube apparatus and were uncorrected. ^{1}H NMR spectra were recorded at 500 MHz 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). Liquid chromatography-mass spectra (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. Chromatographic solvent proportions are indicated as volume:volume ratio. Thin-layer chromatography (TLC) was run using Analtech silica gel GF254 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 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 30% CH_3CN/70% 20 mM H_3PO_4; flow rate 2.0 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 30% CH_3CN/70% 20 mM H_3PO_4; 6.0 mL/min flow rate; 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) 3-(5-Formylfuran-2-yl)benzoic acid (**1**)*: To a mixture of 3-carboxybenzeneboronic acid (2.0 g, 12.05 mmol), 5-bromo-2-furaldehyde (2.11 g, 12.05 mmol), $Pd(OAc)_2$ (135.3 mg, 0.60 mmol) and triphenyl phosphine (632.0 mg, 2.41 mmol) in toluene/EtOH (1:1, 72 mL) was added aqueous 2 M Na_2CO_3. After the reaction mixture was heated at reflux overnight, it was cooled to room temperature (RT). The mixture was diluted with water, washed with EtOAc. The aqueous layer was acidified with aqueous 6 M HCl to pH 1-2. The precipitate was collected by filtration, washed with water and dried *in vacuo* to afford compound **1** as an orange solid (2.31 g, 89%). ^{1}H NMR (DMSO-d_6): δ 13.30 (br s, 1H), 9.66 (s, 1H), 8.39 (s, 1H), 8.13 (d, J = 7.5 Hz, 1H), 8.00 (d, J = 7.5 Hz, 1H), 7.69-7.64 (m, 2H), 7.43 (d, J = 3.5 Hz, 1H). LC-MS (ESI, *m/z*): Calcd for $C_{12}H_7O_4$ ($[M-H]^-$) 215.0, found 215.1.

*(c) 5-(3-(4-Methyl-1,4-diazepane-1-carbonyl)phenyl)furan-2-carbaldehyde (**2a**):* To a stirred solution of compound **1** (150 mg, 0.69 mmol) in anhydrous DMF (4 mL) was added HBTU (394 mg, 1.04 mmol), DIPEA (0.36 mL, 2.08 mmol) under N_2 atmosphere. After the mixture was stirred at RT for 20 min, *N*-methylhomopiperazine (0.13 mL, 1.04 mmol) was added. The reaction mixture was stirred overnight. Water (15 mL) was added to quench the reaction, and the mixture was extracted with butanol. The combined organic layer was washed with water, brine and dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by preparative TLC plates with CH_2Cl_2/MeOH (75:4) as eluent to afford compound **2a** as an orange solid (163 mg, 75%). ^{1}H NMR ($CDCl_3$): δ 9.54 (s, 1H), 7.77-7.73 (m, 2H), 7.38 (t, J = 7.5 Hz, 1H), 7.30 (d, J = 6.5 Hz, 1H), 7.25 (d, J = 4.0 Hz, 1H), 6.81 (s, 1H), 3.72-3.68 (m, 2H), 3.44 (br m, 1H), 3.38 (t, J = 6.0Hz, 1H), 2.68 (br m, 1H), 2.56 (t, J = 5.0 Hz, 1H), 2.49 (br m, 2H), 2.31, 2.25 (s+s, 3H), 1.91 (br m, 1H), 1.76 (br m, 1H). LC-MS (ESI, *m/z*): Calcd for $C_{18}H_{21}N_2O_3$ ($[M+H]^+$) 313.1, found 313.0.

*(d) tert-Butyl 4-(3-(5-formylfuran-2-yl)benzoyl)-1,4-diazepane-1-carboxylate (**2b**):* To a stirred solution of compound **1** (200 mg, 0.93 mmol) in anhydrous DMF (5 mL) was added HBTU (524 mg, 1.38 mmol), DIPEA (0.48 mL, 2.78 mmol) under N_2 atmosphere. After the mixture was stirred at RT for 20 min, 1-Boc-hexahydro-1,4-diazapine (0.27 mL, 1.38 mmol) was added. The reaction mixture was stirred overnight. Water (20 mL) was added to quench the reaction, and the mixture was extracted with CH_2Cl_2. The combined organic layer was washed with water, brine and dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by preparative TLC plates with CH_2Cl_2/MeOH (10:1) as eluent to afford compound **2b** as an orange oil (235 mg, 64%). ^{1}H NMR ($CDCl_3$): δ 9.55 (s, 1H), 7.76 (s, 2H), 7.39 (s, 1H), 7.29-7.25 (m, 2H), 6.88, 6.81 (s+s, 1H), 3.73-3.35 (m, 8H), 1.88 (br m, 1H), 1.61 (br m, 1H), 1.39 (s, 9H). LC-MS (ESI, *m/z*): Calcd for $C_{22}H_{26}N_2O_5Na$ ($[M+Na]^+$) 421.2, found 421.1.

*(e) (E)-3-(5-((5-Chloro-2-oxoindolin-3-ylidene)methyl)furan-2-yl)benzoic acid (**3**):* To a stirred suspension of compound **1** (1.5 g, 6.94 mmol) and 5-chlorooxindole (1.4 g, 8.33 mmol) in anhydrous EtOH (20 mL) was added piperidine (0.82 mL, 8.33 mmol) dropwise under N_2 atomsphere. After the reaction mixture was heated and stirred at 35 ^{o}C for 4 h, it was allowed to stir at RT overnight. The solid was collected by filtration, washed with cold EtOH and hexanes to afford compound **3** as an orange solid (1.78 g, 70%). ^{1}H NMR (DMSO-d_6): δ 13.19 (br s, 1H), 10.72 (s, 1H), 8.51 (s, 1H), 8.45 (s, 1H), 8.17 (d, J = 6.5 Hz, 1H), 8.00 (d, J = 6.0 Hz, 1H), 7.68 (s, 1H), 7.49 (s, 2H), 7.41 (s, 1H), 7.32 (d, J = 7.0 Hz, 1H), 6.91 (d, J = 7.0 Hz, 1H). LC-MS (ESI, *m/z*): Calcd for $C_{20}H_{11}ClNO_4$ ($[M-H]^-$) 364.0, found 364.0.

*(f) (E)-5-Chloro-3-((5-(3-(4-methyl-1,4-diazepane-1-carbonyl)phenyl)furan-2-yl)methylene)indolin-2-one (CX-6258, **4a**):* Method A. To a stirred suspension of compound **2a** (90 mg, 0.29 mmol) and 5-chlorooxindole (51 mg, 0.30 mmol) in anhydrous EtOH (3 mL) was added piperidine (0.1 mL, 1.0 mmol) dropwise under N_2 atomsphere. After the reaction mixture was heated at reflux for 5 h, the solvent was removed *in vacuo.* The residual was purified with preparative TLC plate solid with CH_2Cl_2/MeOH (10:1) as eluent to afford compound **4a** as an orange solid (59 mg, 44%).^{1}H NMR (DMSO-d_6): δ 10.74 (s, 1H), 8.53 (s, 1H), 8.03 (d, J = 8.0 Hz, 1H), 7.94 (s, 1H), 7.61 (t, J = 8.0 Hz, 1H), 7.50 (s, 2H), 7.44-7.42 (m, 2H), 7.33 (d, J = 8.0 Hz, 1H), 6.92 (d, J = 8.5 Hz, 1H), 3.64-3.63 (m, 2H), 3.41-3.38 (m, 2H), 2.65 (br m, 1H), 2.55 (br m, 1H), 2.51-2.47 (m, 2H), 2.29, 2.18 (s+s, 3H), 1.88 (br m, 1H), 1.73 (br m, 1H). LC-MS (ESI, *m/z*): Calcd for $C_{26}H_{25}ClN_3O_3$ ($[M+H]^+$) 462.2, found 462.2. Method B. To a stirred solution of compound **3** (150 mg, 0.41 mmol) in anhydrous DMF (4 mL) was added CDI (133 mg, 0.82 mmol) under N_2 atmosphere. After the mixture was stirred at RT overnight, *N*-methylhomopiperazine (0.13 mL, 1.04 mmol) was added. The reaction mixture was stirred for 24 h. Water (12 mL) was

added to quench the reaction, and the mixture was stirred for 1 h. The precipitate was collected by filtration, washed with water, dried *in vacuo*. The crude product was purified with preparative TLC plates with CH_2Cl_2/MeOH (25:3) as eluent to afford compound **4a** as an orange solid (148.4 mg, 78%). Analytical data was identical with Method A.

*(g) tert-Butyl (E)-4-(3-(5-((5-chloro-2-oxoindolin-3-ylidene)methyl)furan-2-yl)benzoyl)-1,4-diazepane-1-carboxylate (**4b**):* Method A. To a stirred suspension of compound **2b** (100 mg, 0.25 mmol) and 5-chlorooxindole (44 mg, 0.26 mmol) in anhydrous EtOH (2.5 mL) was added piperidine (0.1 mL, 1.0 mmol) dropwise under N_2 atomsphere. After the reaction mixture was heated at reflux for 5 h, the solvent was removed *in vacuo*. The residual was purified with preparative TLC plate solid with CH_2Cl_2/MeOH (50:3) as eluent to afford compound **4b** as an orange solid (137 mg, 64%). ^{1}H NMR ($CDCl_3$): δ 8.77 (s, 1H), 8.58, 8.55 (s+s, 1H), 7.82 (d, J = 7.0 Hz, 1H), 7.51 (quin, J = 7.5 Hz, 1H), 7.38-7.34 (m, 2H), 7.17 (dd, J = 8.0, 17.0 Hz, 1H), 6.82, 6.77 (d+d, J = 8.0, 8.0 Hz, 1H), 3.83 (br m, 1H), 3.74 (t, J = 5.5 Hz, 1H), 3.66 (br m, 1H), 3.57-3.39 (m, 5H), 1.99 (br m, 1H), 1.73 (m, 1H), 1.50 (s, 9H). LC-MS (ESI, *m/z*): Calcd for $C_{30}H_{29}ClN_3O_5$ ($[M-H]^-$) 546.2, found 546.2. Method B. To a stirred solution of compound **3** (200 mg, 0.55 mmol) in anhydrous DMF (5 mL) was added CDI (178 mg, 1.1 mmol) under N_2 atmosphere. After the mixture was stirred at RT overnight, 1-Boc-hexahydro-1,4-diazapine (0.27 mL, 1.38 mmol) was added. The reaction mixture was stirred for 24 h. Water (15 mL) was added to quench the reaction, and the mixture was stirred for 1 h. The precipitate was collected by filtration, washed with water, dried *in vacuo*. The crude product was purified with preparative TLC plates with CH_2Cl_2/MeOH (50:3) as eluent to afford compound **4b** as an orange solid (300 mg, 79%). Analytical data was identical with Method A.

*(h) (E)-3-((5-(3-(1,4-Diazepane-1-carbonyl)phenyl)furan-2-yl)methylene)-5-chloroindolin-2-one (N-desmethyl-CX-6258, **5**):* To a stirred solution of compound **4b** (80 mg, 0.15 mmol) in CH_2Cl_2 (1 mL) was added TFA (0.2 mL) dropwise at 0 °C. After the reaction mixture was stirred for 4 h, the solvent was removed *in vacuo*. The crude product was washed with Et_2O to afford compound **5** TFA salt as an orange solid (54 mg, 66%). ^{1}H NMR (DMSO-d_6): δ 10.76 (s, 1H), 9.00 (s, 2H), 8.54 (s, 1H), 8.06 (d, J = 7.5 Hz, 1H), 7.97 (s, 1H), 7.66 (t, J = 7.0 Hz, 1H), 7.55-7.42 (m, 2H), 7.34 (d, J = 7.5 Hz, 1H), 6.93 (d, J = 8.0 Hz, 1H), 3.86-3.63 (m, 3H), 3.45-3.44 (m, 2H), 3.25 (m, 3H), 2.07-1.95 (m, 2H). LC-MS (ESI, *m/z*): Calcd for $C_{25}H_{23}ClN_3O_3$ ($[M+H]^+$) 448.1, found 448.1. The free base was prepared by addition of compound **5** TFA salt in water and basified with NH_4OH to pH 10. The mixture was extracted with CH_2Cl_2. The combined organic layer was washed with water, brine and dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to afford an orange solid for labelling.

*(i) (E)-5-Chloro-3-((5-(3-(4-[^{11}C]methyl-1,4-diazepane-1-carbonyl)phenyl)furan-2-yl)methylene)indolin-2-one ([^{11}C]CX-6258, [^{11}C]**4a**):* [^{11}C]CO_2 was produced by the $^{14}N(p,\alpha)^{11}C$ 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. Typical irradiations used for the development were 55 μA beam current and 30 min on target. The production run produced approximately 45.5 GBq of [^{11}C]CO_2 at EOB. In a small reaction vial (5 mL), the precursor **5** (0.3-0.5 mg) was dissolved in DMSO (400 μL). To this solution was added NaH (1 mg). No carrier-added (high specific activity) [^{11}C]CH_3OTf that was produced by the gas-phase production method[25] from [^{11}C]CO_2 through [^{11}C]CH_4 and [^{11}C]CH_3Br with silver triflate (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. The contents of the reaction vial were diluted with $NaHCO_3$ solution (0.1 M, 1 mL), and injected onto the semi-preparative RP 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]**4a**. The eluted product was then sterile-filtered through a sterile vented Millex-FG 0.2 μm filter, and collected into a sterile vial. Total radioactivity (4.6-8.2 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** = 3.38 min, t_R **4a** = 5.51 min, t_R [^{11}C]**4a** = 5.64 min. Retention times in the semi-preparative HPLC system were: t_R **5** = 5.83 min, t_R **4a** = 8.67 min, t_R [^{11}C]**4a** = 8.88 min. The radiochemical yield of [^{11}C]**4a** was 40-50% decay corrected to EOB, based on [^{11}C]CO_2.