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Synthesis and *in vitro* biological evaluation of new P2X7R radioligands [^{11}C] halo-GSK1482160 analogs

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ABSTRACT

The reference standards halo-GSK1482160 (F-, Br-, and I-) and their corresponding precursors desmethyl-halo-GSK1482160 (F-, Br-, and I-) were synthesized from (*S*)-1-methyl-5-oxopyrrolidine-2-carboxylic acid or (*S*)-5-oxopyrrolidine-2-carboxylic acid and 2-halo-3-(trifluoromethyl)benzylamine (F-, Br-, and I-) in one step with 45–93% yields. The target tracers [^{11}C]halo-GSK1482160 (F-, Br-, and I-) were prepared from desmethyl-halo-GSK1482160 (F-, Br-, and I-) with [^{11}C]CH_3OTf under basic conditions (NaOH-Na_2CO_3, solid, w/w 1:2) through *N*-[^{11}C]methylation and isolated by HPLC combined with SPE in 40–50% decay corrected radiochemical yield. The radiochemical purity was > 99%, and the molar activity (A_M) at end of bombardment (EOB) was 370–740 GBq/μmol. The potency of halo-GSK1482160 (F-, Br-, and I-) in comparison with GSK1482160 (Cl-) was determined by a radioligand competitive binding assay using [^{11}C]GSK1482160, and the binding affinity K_i values for halo-GSK1482160 (F-, Br-, and I-) and GSK1482160 (Cl-) are 54.2, 2.5, 1.9 and 3.1 nM, respectively.

The purinergic receptor P2X ligand-gated ion channel type 7 (P2X7R) is an adenosine triphosphate (ATP)-gated ion-channel, and P2X7R is a key player in inflammation.[1] P2X7R is an emerging therapeutic target in central nervous system (CNS) diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD), because the overexpression of P2X7R is closely associated with neuroinflammation.[2] P2X7R has become a novel molecular imaging target for neuroinflammation via biomedical imaging technique positron emission tomography (PET).[3] Recently several radioligands targeting P2X7R have been developed and evaluated in animals and human, and the representative radioligands are shown in Fig. 1.[4–15] However, biological evaluation indicated some of these P2X7R radioligands have significant drawbacks like not potent enough binding affinity K_i values, short half-life of radionuclide carbon-11 ($t_{1/2}$, 20.4 min), limited blood-brain barrier (BBB) penetration and/or little brain uptake. Thus an ideal P2X7R radioligand that can be used in the clinical setting to study P2X7R expression levels in neurodegenerative disorders remains to be discovered. In our previous work, we have developed and characterized [^{11}C]GSK1482160 as a P2X7R radioligand for neuroinflammation,[7,8] clinical evaluation of [^{11}C]GSK1482160 in healthy controls and patients is currently underway, the estimation of radiation dosimetry for [^{11}C]GSK1482160 in normal human subjects has been reported, and the results indicated brain uptake was low, but in most other organs the uptake and clearance of [^{11}C]GSK1482160 appears suitable for use in PET assessment of P2X7R expression as a potential marker of regional inflammation.[15] In addition, a series of [^{18}F]fluoroalkyl derivatives of GSK1482160 like [^{18}F]fluoroethyl derivative [^{18}F]IUR-1601 and [^{18}F]fluoropropyl derivative [^{18}F]IUR-1602 have been also developed in this laboratory.[12,13] Although these two fluorine-18 radioligands has a longer half-life ($t_{1/2}$, 109.7 min) and greater potential for widespread clinical use, they requires complicated two-step radiosynthetic procedures and are unstable under predisposing radiosynthetic conditions for an elimination reaction, resulting low radiochemical yield.[12,13] To overcome the problems with [^{11}C]GSK1482160 (short half-life and low brain uptake) and with [^{18}F]IUR-1601 as well as [^{18}F]IUR-1602 (complex radiosynthesis and short-term stability), in this ongoing study, we initially proposed to develop [^{18}F]F-GSK1482160, in which GSK1482160 and its bromine and iodine analogs serve as the labeling precursors for [^{18}F]F-GSK1482160, but the *in vitro* biological evaluation results of halo-GSK1482160 (F-, Cl-, Br-, and I-) analogs suggest [^{18}F]F-GSK1482160 is not a useful radioligand, instead [^{11}C]Br-GSK1482160 and [^{11}C]I-GSK1482160 could be useful radioligands. Therefore, we turned our effort to develop [^{11}C]halo-GSK1482160 (F-, Br-, and I-) analogs. Herein, we report the design, synthesis, radiolabeling and initial *in vitro* biological evaluation of new P2X7R radioligands [^{11}C]halo-GSK1482160 (F-, Br-, and I-) analogs (Fig. 1).

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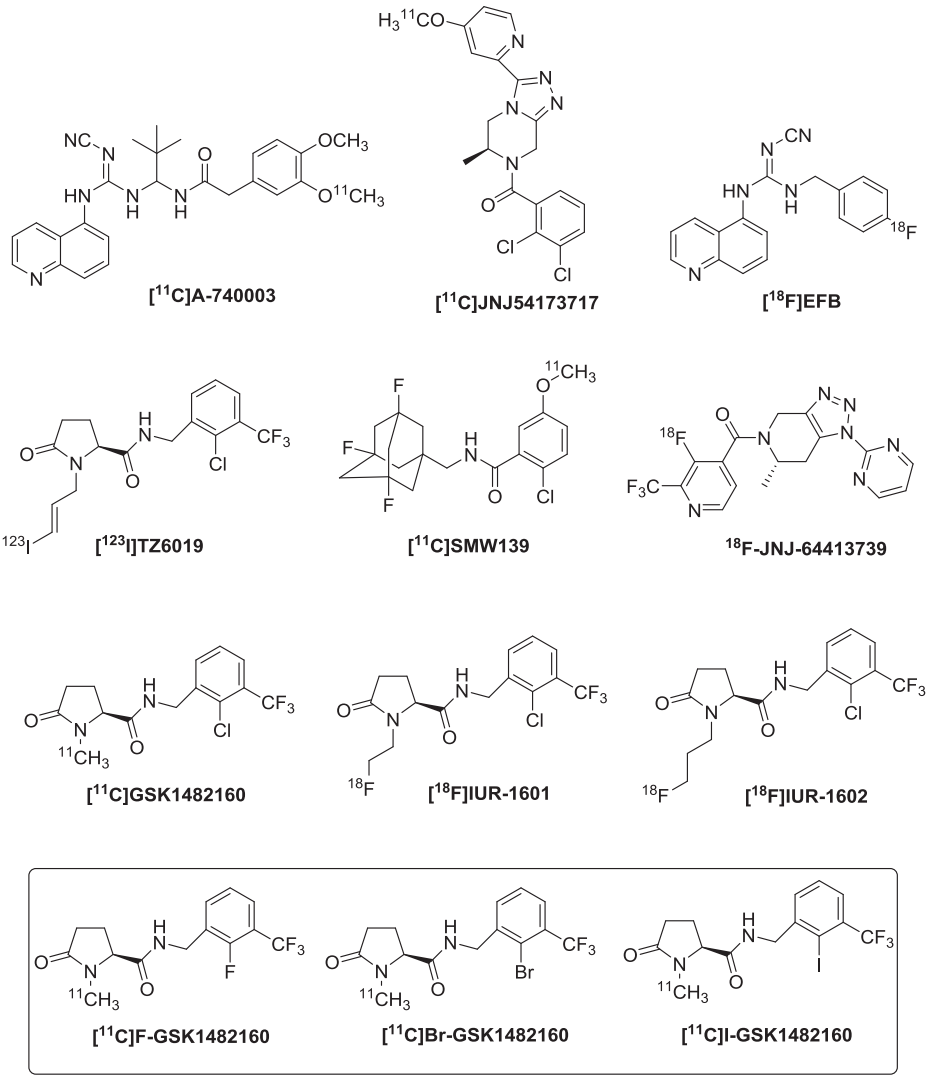
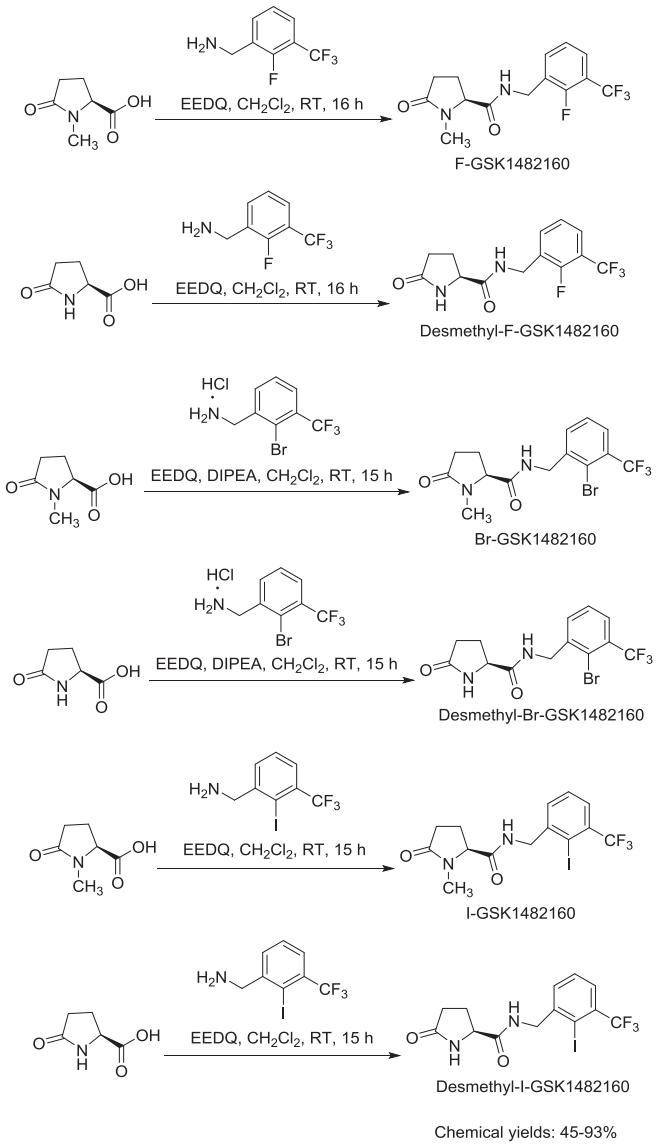


Figure 1. Radioligands for imaging of P2X7R.

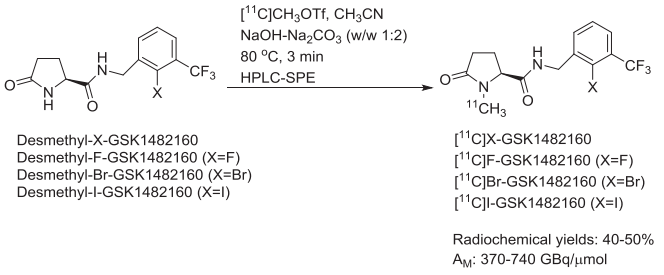
The reference standards: F-GSK1482160 ((*S*)-*N*-(3-fluoro-4-(trifluoromethyl)benzyl)-1-methyl-5-oxopyrrolidine-2-carboxamide), Br-GSK1482160 ((*S*)-*N*-(3-bromo-4-(trifluoromethyl)benzyl)-1-methyl-5-oxopyrrolidine-2-carboxamide), I-GSK1482160 ((*S*)-*N*-(3-iodo-4-(trifluoromethyl)benzyl)-1-methyl-5-oxopyrrolidine-2-carboxamide); and their corresponding precursor: desmethyl-F-GSK1482160 ((*S*)-*N*-(3-fluoro-4-(trifluoromethyl)benzyl)-5-oxopyrrolidine-2-carboxamide), desmethyl-Br-GSK1482160 ((*S*)-*N*-(3-bromo-4-(trifluoromethyl)benzyl)-5-oxopyrrolidine-2-carboxamide), desmethyl-I-GSK1482160 ((*S*)-*N*-(3-iodo-4-(trifluoromethyl)benzyl)-5-oxopyrrolidine-2-carboxamide); were synthesized as outlined in Scheme 1, according to the published methods.[7] The starting materials were either commercially available or synthesized in our previous work.[7] (*S*)-1-Methyl-5-oxopyrrolidine-2-carboxylic acid underwent a coupling reaction with 2-halo-3-(trifluoromethyl)benzylamine (F-, Br-, and I-) using 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) as a catalyst, affording halo-GSK1482160 (F-, Br-, and I-). Likewise, (*S*)-5-oxopyrrolidine-2-carboxylic acid underwent a coupling reaction with 2-halo-3-(trifluoromethyl)benzylamine (F-, Br-, and I-) using EEDQ as a catalyst, giving desmethyl-halo-GSK1482160 (F-, Br-, and I-). The yields of the coupling reaction were in the range of 45–93%. F-GSK1482160 appeared in the GlaxoSmithKline patents without biological activity data,[16,17] Br-GSK1482160, I-GSK1482160, and all three desmethylated precursors are new compounds.

Synthesis of the target tracers [^{11}C]F-GSK1482160 ((*S*)-*N*-(3-fluoro-4-(trifluoromethyl)benzyl)-1-[^{11}C]methyl-5-oxopyrrolidine-2-carboxamide), [^{11}C]Br-GSK1482160 ((*S*)-*N*-(3-bromo-4-(trifluoromethyl)benzyl)-1-[^{11}**C**]methyl-5-oxopyrrolidine-2-carboxamide), and [^{11}C]I-GSK1482160 ((*S*)-*N*-(3-iodo-4-(trifluoromethyl)benzyl)-1-[^{11}C]methyl-5-oxopyrrolidine-2-carboxamide) is presented in Scheme 2. Desmethyl-halo-GSK1482160 (F-, Br-, and I-) underwent *N*-[^{11}C]methylation[7] using the reactive [^{11}C]methylating agent [^{11}C]methyl triflate ([^{11}C]CH_3OTf)[18,19] in acetonitrile at 80 °C under basic conditions (NaOH-Na_2CO_3, solid, w/w 1:2). The product was isolated by semi-preparative reverse-phase (RP) high performance liquid chromatography (HPLC) with a C-18 column, and then concentrated by solid-phase extraction (SPE) with a disposable C-18 Plus Sep-Pak cartridge to produce the corresponding pure radiolabeled compound [^{11}C]halo-GSK1482160 (F-, Br-, and I-) in 40–50% radiochemical yield, decay corrected to end of bombardment (EOB), based on [^{11}C]CO_2.

Desmethyl-halo-GSK1482160 (F-, Br-, and I-) contain both cyclic amide and side chain amide that can be *N*-[^{11}C]methylated. They are two competing reactions. Although the cyclic amide is more easily deprotonated and methylated than side chain amide to produce desired [^{11}C]halo-GSK1482160 (F-, Br-, and I-), there was always a radiolabeled [^{11}C]halo-GSK1482160 isomer (F-, Br-, and I-) by-product [^{11}C]-methylated at side chain amide position formed. During the development, validation and production of [^{11}C]GSK1482160,[7] We



Scheme 1. Synthesis of the reference standards halo-GSK1482160 (F-, Br-, and I-) and their corresponding precursor desmethyl-halo-GSK1482160 (F-, Br-, and I-).



Scheme 2. Synthesis of the target tracers [^{11}C]halo-GSK1482160 (F-, Br-, and I-).

surprisingly found out [^{11}C]GSK1482160 and its radiolabeled isomer were formed in different ratio using the old NaH base or freshly purchased NaH base. For old NaH used in development and optimization runs, [^{11}C]GSK1482160 was formed as a major labeled product; but for freshly purchased NaH (expired in one year) used in validation and production runs, undesired [^{11}C]GSK1482160 isomer was formed as a major labeled product. The reason could be that old NaH might have absorbed moisture to become NaOH and/or reacted with CO_2 in the air to become $NaHCO_3$, a solid mixture of NaOH-Na_2CO_3. It is likely the different pKa value of cyclic amide and side chain amide requires different base for *N*-[^{11}C]methylation. Consequently we investigated the base effect on the radiosynthesis of [^{11}C]GSK1482160 and its halo-analogs. The results are shown in Scheme 3. If NaOH-Na_2CO_3 (w/w 1:2, solid) was used as a base, [^{11}C]halo-GSK1482160 (F-, Br-, and I-) and [^{11}C]halo-GSK1482160 isomer (F-, Br-, and I-) were formed in a ~ 10:1 ratio. If NaOH (solid) was used as a base, [^{11}C]halo-GSK1482160 (F-, Br-, and I-) and [^{11}C]halo-GSK1482160 isomer (F-, Br-, and I-) were formed in a ~1:1 ratio. If NaH (95% dry or 60% dispersion in mineral oil, powder) was used as a base, [^{11}C]halo-GSK1482160 (F-, Br-, and I-) and [^{11}C]halo-GSK1482160 isomer (F-, Br-, and I-) were formed in a ~1:10 ratio.

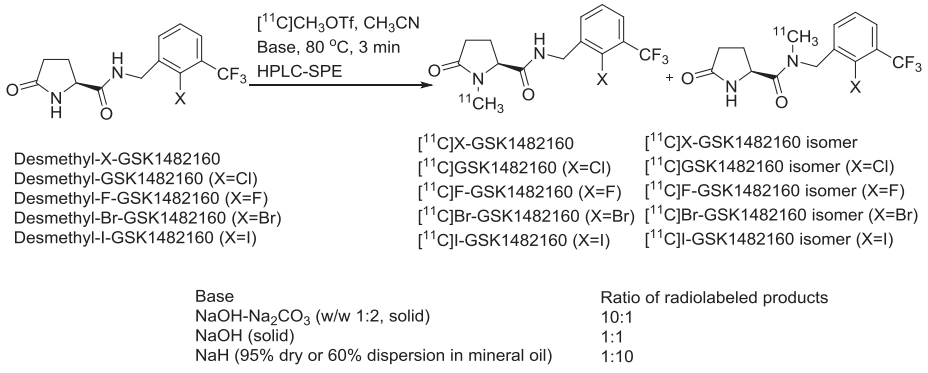
The radiosynthesis was performed in a home-built automated multi-purpose [^{11}C]-radiosynthesis module.[20–22] Our radiosynthesis module facilitated the overall design of the reaction, purification and re-formulation capabilities in a fashion suitable for adaptation to preparation of human doses. The radiosynthesis includes three stages: 1) labeling reaction; 2) purification; and 3) formulation. The overall synthesis time was ~40 min from EOB. Our module is also designed to allow in-process measurement of [^{11}C]-tracer molar activity (A_M, GBq/μmol at EOB) using a radiation detector with a UV detector at the outlet of the HPLC-portion of the system. At the end of synthesis (EOS), the A_M of [^{11}C]-tracer was determined again by analytical RP-HPLC, calculated, decay corrected to EOB, and based on [^{11}C]CO_2, which was in agreement with the 'on line' determined value. The A_M of [^{11}C]halo-GSK1482160 analogs at EOB was 370–740 GBq/μmol.

Chemical purity and radiochemical purity were determined by analytical HPLC.[23] The chemical purity of the precursor and reference standard was > 95% determined by RP-HPLC through UV flow detector. The radiochemical purity of the target tracer was > 99% determined by radio-HPLC through γ-ray (PIN diode) flow detector.

The preliminary biological evaluation of halo-GSK1482160 (F-, Br-, and I-) in comparison with the known P2X7R ligand GSK1482160 was performed by a radioligand competitive binding assay using [^{11}C]GSK1482160 following the literature method.[8,12,13,24–26] The results are shown in Figure 2. [^{11}C]GSK1482160 was used as the radioligand, GSK1482160 and buffer were used as a positive control and a negative control, respectively. All data were analyzed by GraphPad Prism 7.00 software using nonlinear regression and the competitive binding model One site – Fit K_i. The binding affinity K_i values for GSK1482160 (F-, Br-, and I-) and GSK1482160 are 54.2 ± 6.2 nM, 2.5 ± 0.7 nM, 1.9 ± 0.6 nM, and 3.1 ± 0.3 nM (n = 3), respectively. The IC_{50} values for GSK1482160 (F-, Br-, and I-) and GSK1482160 are 152.6 ± 17.5 nM, 7.1 ± 1.9 nM, 5.3 ± 1.5 nM, and 8.9 ± 1.0 nM (n = 3), respectively, also obtained via GraphPad Prism 7.00 software using nonlinear regression and the competitive binding model One site – Fit logIC_{50}.

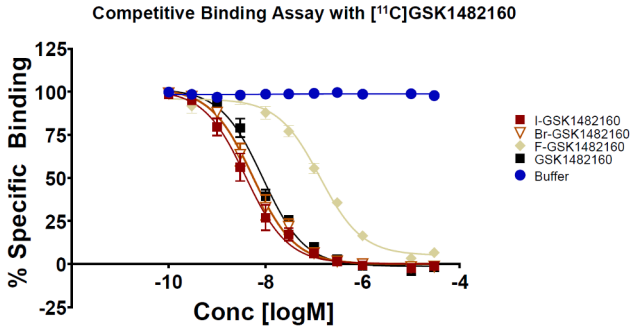
The experimental details and characterization data for halo-GSK1482160 (F-, Br-, and I-), desmethyl-halo-GSK1482160 (F-, Br-, and I-), and [^{11}C]halo-GSK1482160 (F-, Br-, and I-), as well as radioligand competitive binding assay are given.[27]

In summary, facile synthetic routes with moderate to excellent yields have been developed to produce the precursors desmethyl-halo-GSK1482160 (F-, Br-, and I-), the reference standards halo-GSK1482160 (F-, Br-, and I-), and the target PET radiotracers [^{11}C]halo-GSK1482160 (F-, Br-, and I-). The radiosynthesis employed [^{11}C]CH_3OTf for *N*-[^{11}C]methylation at the nitrogen position of the amide precursors, followed by product purification and isolation by a semi-preparative RP-HPLC combined with SPE. The base effect on the radiotracer production of [^{11}C]GSK1482160 and [^{11}C]halo-GSK1482160 (F-, Br-, and I-) has been investigated. The desired [^{11}C]halo-GSK1482160 (F-, Br-, and I-) were obtained in high radiochemical yield, and radiochemical purity, with a reasonably short overall synthesis time, and high molar activity. New P2X7R radioligands [^{11}C]



Base	Ratio of radiolabeled products
NaOH-Na_2CO_3 (w/w 1:2, solid)	10:1
NaOH (solid)	1:1
NaH (95% dry or 60% dispersion in mineral oil)	1:10

Scheme 3. Base effect on the radiosynthesis of [^{11}C]GSK1482160 and its halo-analogs.



Compound	GSK1482160	F-GSK1482160	Br-GSK1482160	I-GSK1482160
K_i (nM)	3.1 ± 0.3	54.2 ± 6.2	2.5 ± 0.7	1.9 ± 0.6
IC_{50} (nM)	8.9 ± 1.0	152.6 ± 17.5	7.1 ± 1.9	5.3 ± 1.5

Figure 2. The result of the competitive binding assay of halo-GSK1482160 (F-, Br-, and I-) in comparison with GSK1482160.

halo-GSK1482160 (F-, Br-, and I-) have been successfully radiosynthesized. The initial *in vitro* biological evaluation results indicate that [^{11}C]F-GSK1482160 has ~17-fold less potency compared to the parent radioligand [^{11}C]GSK1482160, but remains low nM grade P2X7R affinity; [^{11}C]Br-GSK1482160 and [^{11}C]I-GSK1482160 display very similar even superior P2X7R affinity to [^{11}C]GSK1482160. These will facilitate studies to evaluate [^{11}C]Br-GSK1482160 and [^{11}C]I-GSK1482160 as new PET radiopharmaceuticals for targeting the $P2X_7$ receptor in animals and humans. The *in vivo* biological evaluation of [^{11}C]Br-GSK1482160 and [^{11}C]I-GSK1482160 is currently underway.

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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. Melting points

were determined on a MEL-TEMP II capillary tube apparatus and were uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance II 500 MHz NMR Fourier transform spectrometer at 500 and 125 MHz, respectively. Chemical shifts (δ) are reported in parts per million (ppm) relative to an internal standard tetramethylsilane (TMS, δ 0.0) (^{1}H NMR) and to the solvent signal (^{13}C NMR), and coupling constants (J) are 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. 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. 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). [^{11}C]GSK1482160 is a routine radiotracer produced in our PET radiochemistry facility. The analytical RP-HPLC system included a Prodigy (Phenomenex) 5 μm C-18 column, 4.6 × 250 mm, mobile phase 35% CH_3CN/65% 20 mM H_3PO_4, flow rate 1.8 mL/min; UV (275 nm) and γ-ray (PIN diode) flow detectors. The semi-preparative RP-HPLC system included a Prodigy (Phenomenex) 5 μm C-18 column, 10 × 250 mm, mobile phase 35% CH_3CN/65% 0.1 M CH_3CO_2Na, flow rate 6, 7, and 8 mL/min for [^{11}C]F-GSK1482160, [^{11}C]Br-GSK1482160, and [^{11}C]I-GSK1482160, respectively; UV (254 nm) and γ-ray (PIN diode) flow detectors. *(b). General synthetic method for F-GSK1482160 ((S)-N-(3-fluoro-4-(trifluoromethyl)benzyl)-1-methyl-5-oxopyrrolidine-2-carboxamide), Br-GSK1482160 ((S)-N-(3-bromo-4-(trifluoromethyl)benzyl)-1-methyl-5-oxopyrrolidine-2-carboxamide), I-GSK1482160 ((S)-N-(3-iodo-4-(trifluoromethyl)benzyl)-1-methyl-5-oxopyrrolidine-2-carboxamide); desmethyl-F-GSK1482160 ((S)-N-(3-fluoro-4-(trifluoromethyl)benzyl)-5-oxopyrrolidine-2-carboxamide), desmethyl-Br-GSK1482160 ((S)-N-(3-bromo-4-(trifluoromethyl)benzyl)-5-oxopyrrolidine-2-carboxamide), desmethyl-I-GSK1482160 ((S)-N-(3-iodo-4-(trifluoromethyl)benzyl)-5-oxopyrrolidine-2-carboxamide):* (*S*)-1-Methyl-5-oxopyrrolidine-2-carboxylic acid or (*S*)-5-oxopyrrolidine-2-carboxylic acid (2.0 mmol) was suspended in CH_2Cl_2 (60 mL), and EEDQ (2.2 mmol) was added. The mixture was stirred at room temperature (RT) for 10 min. A solution of 2-fluoro-3-(trifluoromethyl)benzylamine (2.1 mmol), 2-bromo-3-(trifluoromethyl) benzylamine HCl salt (2.1 mmol) with *N*,*N*-diisopropylethylamine (DIPEA, 3.0 mmol), or 2-iodo-3-(trifluoromethyl)benzylamine (2.1 mmol) in CH_2Cl_2 (20 mL) was then added dropwise to the mixture. The mixture was then stirred at RT for 15-16 h. The reaction was monitored by TLC. The mixture was washed with saturated aqueous $NaHCO_3$ (40 mL), 2 N aqueous HCl (40 mL × 2), and water. The organic solution was dried over Na_2SO_4, filtered and concentrated in vacuo. The resulting crude product was then purified by column chromatography on silica gel with eluent (3-7% MeOH/CH_2Cl_2) to obtain white solid product (45-93%). **F-GSK1482160**: Mp 111-112 °C. ^{1}H NMR (acetone-d_6): δ 1.99-2.04 (m, 1H, CH), 2.30-2.36 (m, 1H, CH), 2.37-2.47 (m, 2H, CH_2), 2.78 (s, 3H, NCH_3), 4.01 (dd, J = 4.0, 4.9 Hz, 1H, CH), 4.56 (d, J = 5.9 Hz, 2H, CH_2), 6.82 (br s, 1H, NH), 7.21 (t, J = 7.7 Hz, 1H, Ph-H), 7.57 (d, J = 7.6 Hz, 2H, Ph-H). MS (ESI): 319 ([M + H]$^+$, 100%). **Br-GSK1482160**: Mp 168-169 °C. ^{1}H NMR ($CDCl_3$): δ 2.03-2.05 (m, 1H, CH), 2.27-2.32 (m, 1H, CH), 2.36-2.39 (m, 1H, CH), 2.42-2.47 (m, 1H, CH), 2.80 (s, 3H, NCH_3), 4.00 (dd, J = 4.0, 4.5 Hz, 1H, CH), 4.62-4.66 (m, 2H, CH_2), 6.88 (br s, 1H, NH), 7.41 (t, J = 5.5 Hz, 1H, Ph-H), 7.57 (d, J = 6.0 Hz, 1H, Ph-H), 7.64 (d, J = 6.5 Hz, 1H, Ph-H). ^{13}C NMR ($CDCl_3$): δ 23.42, 29.22, 29.42, 44.44, 63.81, 121.51, 122.81 (q, $J_{C\text{-}F}$ = 271.9 Hz, CF_3), 127.48 (q, $J_{C\text{-}F}$ = 5.4 Hz), 127.61, 131.21 (d, $J_{C\text{-}F}$ = 30.6 Hz), 133.86, 139.52, 171.50, 175.94. HRMS (ESI): calcd for $C_{14}H_{15}N_2O_2BrF_3$ ([M + H]$^+$) 379.0264, found 379.0267. **I-GSK1482160**: Mp 175-176 °C. ^{1}H NMR ($CDCl_3$): δ 2.02-2.07 (m, 1H, CH), 2.30-2.41 (m, 2H, CH_2), 2.43-2.51 (m, 1H, CH), 2.83 (s, 3H, NCH_3), 4.01 (dd, J = 3.5, 5.0 Hz, 1H, CH), 4.61-4.64 (m, 2H, CH_2), 6.97 (br s, 1H, NH), 7.44 (t, J = 5.0 Hz, 1H, Ph-H), 7.55 (d, J = 7.5 Hz, 1H, Ph-H), 7.61 (d, J = 7.5 Hz, 1H, Ph-H). ^{13}C NMR ($CDCl_3$): δ 23.41, 29.30, 29.44, 49.56, 63.80, 96.63, 122.92 (q, $J_{C\text{-}F}$ = 272.4 Hz, CF_3), 127.22 (q, $J_{C\text{-}F}$ = 5.9 Hz), 128.49, 132.98, 134.89 (d, $J_{C\text{-}F}$ = 30.5 Hz), 142.95, 171.40, 175.87. HRMS (ESI): calcd for $C_{14}H_{15}N_2O_2IF_3$ ([M + H]$^+$) 427.0125, found 427.0125. **Desmethyl-F-GSK1482160**: Mp 109-110 °C. ^{1}H NMR (acetone-d_6): δ 2.12-2.17 (m, 1H, CH), 2.22-2.34 (m, 2H, CH_2), 2.45-2.53 (m, 1H, CH), 4.19 (dd, J = 4.5, 5.9 Hz, 1H, CH), 4.47-4.56 (m, 2H, CH_2), 7.19 (br s, 1H, NH), 7.21 (dd, J = 3.8, 5.6 Hz, 2H, Ph-H), 7.51 (t, J = 6.9 Hz, 1H, Ph-H), 7.56 (br s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 25.66, 29.22, 36.95, 57.14, 118.40 (dd, $J_{C\text{-}F}$ = 12.5, 32.5 Hz), 122.52 (q, $J_{C\text{-}F}$ = 271.2 Hz, CF_3), 124.13 (d, $J_{C\text{-}F}$ = 5.0 Hz), 126.45 (d, $J_{C\text{-}F}$ = 5.0 Hz), 126.76 (d, $J_{C\text{-}F}$ = 137.5 Hz), 134.21 (d, $J_{C\text{-}F}$ = 3.7 Hz), 157.84 (d, $J_{C\text{-}F}$ = 2.5 Hz), 172.66, 179.75. HRMS (ESI): calcd for $C_{13}H_{13}N_2O_2F_4$ ([M + H]$^+$) 305.0908, found 305.0911. **Desmethyl-Br-GSK1482160**: Mp 190-191 °C. ^{1}H NMR ($CDCl_3$): δ 2.02-2.05 (m, 1H, CH), 2.28-2.32 (m, 1H, CH), 2.36-2.40 (m, 1H, CH), 2.42-2.47 (m, 1H, CH), 4.01 (dd, J = 4.0, 4.5 Hz, 1H, CH), 4.53-4.63 (m, 2H, CH_2), 6.88 (br s, 1H, NH), 7.41 (dd, J = 5.5, 7.0 Hz, 1H, Ph-H), 7.57 (d, J = 6.0 Hz, 1H, Ph-H), 7.64 (d, J = 6.5 Hz, 1H, Ph-H), 7.69 (br s, 1H, NH). ^{13}C NMR ($CDCl_3$ + CD_3OD): δ 25.49, 29.24, 43.95, 56.85, 121.10, 122.84 (q, $J_{C\text{-}F}$ = 272.5 Hz, CF_3), 127.02 (q, $J_{C\text{-}F}$ = 5.8 Hz), 127.35, 130.84 (d, $J_{C\text{-}F}$ = 30.8 Hz), 132.80, 139.41, 172.59, 179.69. HRMS (ESI): calcd for $C_{13}H_{13}N_2O_2BrF_3$ ([M + H]$^+$) 365.0107, found 365.0111. **Desmethyl-I-GSK1482160**: Mp 195-196 °C. ^{1}H NMR (acetone-d_6): δ 2.13-2.19 (m, 2H, CH_2), 2.20-2.32 (m, 1H, CH), 2.44-2.51 (m, 1H, CH), 4.24 (dd, J = 3.0, 5.0 Hz, 1H, CH), 4.50-4.60 (m, 2H, CH_2), 7.07 (br s, 1H, NH), 7.54 (t,J = 7.5 Hz, 1H, Ph-H), 7.61 (d, J = 7.5 Hz, 1H, Ph-H), 7.64 (d, J = 7.5 Hz, 1H, Ph-H), 7.96 (br s, 1H, NH). ^{13}C NMR ($CDCl_3$ + CD_3OD): δ 25.33, 29.16, 49.30, 56.70, 95.89, 122.78 (q, $J_{C\text{-}F}$ = 272.1 Hz, CF_3), 126.63 (q, $J_{C\text{-}F}$ = 5.8 Hz), 128.09, 131.61, 134.40 (d, $J_{C\text{-}F}$ = 30.0 Hz), 142.58, 172.63, 179.66. HRMS (ESI): calcd for $C_{13}H_{13}N_2O_2IF_3$ ([M + H]$^+$) 412.9968, found 412.9970. *(c). General radiosynthetic method for [^{11}C]F-GSK1482160 ((S)-N-(3-fluoro-4-(trifluoromethyl)benzyl)-1-[^{11}C]methyl-5-oxopyrrolidine-2-carboxamide), [^{11}C]Br-GSK1482160 ((S)-N-(3-bromo-4-(trifluoromethyl)benzyl)-1-[^{11}C]methyl-5-oxopyrrolidine-2-carboxamide), and [^{11}C]I-GSK1482160 ((S)-N-(3-iodo-4-(trifluoromethyl)benzyl)-1-[^{11}C]methyl-5-oxopyrrolidine-2-carboxamide):* [^{11}C]CO_2 was produced by the ^{14}N(p,α)^{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 desmethylated precursor 4 (0.3-0.5 mg) was dissolved in CH_3CN (500 μL). To this solution was added NaOH-Na_2CO_3 (solid, w/w 1:2, 1 mg). No carrier-added (high molar activity) [^{11}C]CH_3OTf that was produced by the gas-phase production method[19] 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] F-GSK1482160**, **[^{11}C] Br-GSK1482160**, or **[^{11}C] I-GSK1482160**. 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 ~40 min from EOB. Retention times in the analytical RP-HPLC system were: t_R desmethyl-F-GSK1482160 = 4.38 min, t_R F-GSK1482160 = 5.55 min, t_R [^{11}C]F-GSK1482160 = 5.61 min; t_R desmethyl-Br-GSK1482160 = 5.75 min, t_R Br-GSK1482160 = 7.45 min, t_R [^{11}C]Br-GSK1482160 = 7.52 min; and t_R desmethyl-I-GSK1482160 = 6.50 min, t_R I-GSK1482160 = 8.55 min, t_R [^{11}C]I-GSK1482160 = 8.63 min. Retention times in the semi-preparative RP-HPLC system were: t_R desmethyl-F-GSK1482160 = 7.25 min, t_R F-GSK1482160 = 9.00 min, t_R [^{11}C]F-GSK1482160 = 9.08 min; t_R desmethyl-Br-GSK1482160 = 9.21 min, t_R Br-GSK1482160 = 12.76 min, t_R [^{11}C]Br-GSK1482160 = 12.82 min; and t_R desmethyl-I-GSK1482160 = 9.17 min, t_R I-GSK1482160 = 12.80 min, t_R [^{11}C]I-GSK1482160 = 12.93 min. The radiochemical yield was 40-50% decay corrected to EOB, based on [^{11}C]CO_2. *(d). Cell culture and membrane preparation:* HEK293 cells expressing human recombinant P2X7 receptor (hP2X7R) were obtained from B'SYS GmbH and cultured according to the supplier's procedure. Cells were grown to 80% confluency and then rinsed with phosphate-buffered saline (PBS), detached with trypsin, and harvested. Cell pellets were obtained by centrifugation at 200 *g* for 5 min at 4 °C. Collected cell pellets were frozen at −80 °C until membrane preparation. For the membrane preparation, pellets from 10 T225 flasks were homogenized in 50 mM Tris-HCl, 5 mM ethylenediaminetetraacetic acid (EDTA), and 140 mM NaCl at pH 7.4 and 4 °C. The homogenate was then centrifuged at 48,000 *g* for 20 min at 4 °C, gently rinsed with deionized water, and then resuspended in 50 mM Tris-HCl at pH 7.4 and 4 °C. This homogenate was then centrifuged as before, with the resulting pellet being homogenized in 50 mM Tris-HCl at pH 7.4. Total protein concentration was determined via the Bradford protein assay (Bio-Rad). Aliquots were stored in cryovials at −80 °C until the day of assay. *(e). [^{11}C]GSK1482160 standards:* For each experiment, the highest concentration of radioactivity used was diluted twice in assay buffer, followed by eleven 2-fold dilutions. Twenty microliters of each dilution were then spotted onto a GF/B UniFilter-96 plate (Perkin-Elmer) and allowed to air-dry until the end of the experiment. An additional 20 μL of each dilution were added to a scintillation vial containing 7 mL of Optiphase Hisafe 3 (Perkin-Elmer) and counted on an LS6000 scintillation counter (Beckman). Aliquots of the working concentrations of radioactivity used on each day were counted in the same manner. *(f). Radioligand competitive binding assay:* For competitive binding assays, cell membrane preparation (0.054 mg of protein/mL of assay medium) was incubated with 11 compound concentrations over a six log unit range, with 5 nM [^{11}C]GSK1482160 in assay buffer (50 mM Tris-HCl, pH 7.4, 0.1% bovine serum albumen (BSA)) . Triplicate determinations were done at each concentration of test compound. GSK1482160 was used to determine non-specific binding. Assays were incubated at 22 °C for 30 min. For termination of the binding reaction, the samples were filtered onto GF/B UniFilter plates that had been presoaked in 0.5% polyethyleneimine for 30 min using a UniFilter-96 cell harvester. The plates were washed 5 times with ice-cold saline, dried under a vacuum, and exposed to a TR2025 phosphor screen for 20–60 min. Phosphor screens were then read on a Typhoon FLA-7000IP (GE Healthcare) along with [^{11}C]-calibration standards. CPMs (counts per minute) were determined by calibrating the image to the CPMs in the calibration standards via MCID analysis Software. Data was analyzed with Prism 7.00 (GraphPad Software Inc., La Jolla, California, USA) to determine K_i and IC_{50} values.