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Synthesis and preliminary biological evaluation of a novel P2X7R radioligand [^{18}F]IUR-1601

Mingzhang Gao, Min Wang, Barbara E. Glick-Wilson, Jill A. Meyer, Jonathan S. Peters, Paul R. Territo, Mark A. Green, Gary D. Hutchins, Hamideh Zarrinmayeh, Qi-Huang Zheng *

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

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ABSTRACT

The reference standard IUR-1601 ((*S*)-*N*-(2-chloro-3-(trifluoromethyl)benzyl)-1-(2-fluoroethyl)-5-oxopyrrolidine-2-carboxamide) was synthesized from *tert*-butyl (*S*)-5-oxopyrrolidine-2-carboxylate, fluoroethylbromide, and 2-chloro-3-(trifluoromethyl)benzylamine with overall chemical yield 12% in three steps. The target tracer [^{18}F]IUR-1601 ((*S*)-*N*-(2-chloro-3-(trifluoromethyl)benzyl)-1-(2-[^{18}F]fluoroethyl)-5-oxopyrrolidine-2-carboxamide) was synthesized from desmethyl-GSK1482160 with 2-[^{18}F]fluoroethyl tosylate, prepared from 1,2-ethylene glycol-bis-tosylate and K[^{18}F]F/Kryptofix2.2.2, in two steps and isolated by HPLC combined with SPE in 1–3% decay corrected radiochemical yield. The radiochemical purity was >99%, and the molar activity at end of bombardment (EOB) was 74–370 GBq/μmol. The potency of IUR-1601 in comparison with GSK1482160 was determined by a radioligand competitive binding assay using [^{11}C]GSK1482160, and the binding affinity K_i values for IUR-1601 and GSK1482160 are 4.31 and 5.14 nM, respectively.

The purinergic receptor P2X ligand-gated ion channel type 7 (P2X7R) is an adenosine triphosphate (ATP)-gated ion-channel, and the overexpression of P2X7R is closely associated with neuroinflammation, which plays an important role in various neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD).[1,2] 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 the representative radioligands are shown in Fig. 1.[4–10] However, preclinical evaluation indicated some of these P2X7R radioligands have significant drawbacks like short half-life, 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] and clinical evaluation of [^{11}C]GSK1482160 in normal human subjects are currently underway. The half-life ($t_{1/2}$) of radionuclide carbon-11, 20.4 min, limits the imaging protocol to a maximum of about 90 min post-injection of a carbon-11 radiopharmaceutical, after that insufficient counts are available for meaningful measurements. If imaging were extended past 90 min, more complete information could be obtained regarding the concentration and localization of the P2X7R levels in the tissue. Therefore, it is attractive for us to develop analogs of [^{11}C]GSK1482160 which can be labeled with the radionuclide fluorine-18 ($t_{1/2}$, 109.7 min). A fluorine-18 ligand would be ideal for widespread use, which permits imaging of up to 5 h post-injection, and will result in a better match between the pharmacokinetics of binding and the physical decay of the label. To this end a series of [^{18}F]fluoroalkyl analogs of GSK1482160 will be considered as new potential P2X7R radioligands. In this ongoing study, we report here the synthesis and preliminary biological evaluation of a new P2X7R radioligand [^{18}F]IUR-1601 ((*S*)-*N*-(2-chloro-3-(trifluoromethyl)benzyl)-1-(2-[^{18}F]fluoroethyl)-5-oxopyrrolidine-2-carboxamide, [^{18}F]**3a**) (Fig. 1).

Our synthetic strategy was first to conduct one-step radiosynthesis of [^{18}F]IUR-1601. The reference standard IUR-1601 ((*S*)-*N*-(2-chloro-3-(trifluoromethyl)benzyl)-1-(2-fluoroethyl)-5-oxopyrrolidine-2-carboxamide, **3a**) and its corresponding precursor Cl-IUR-1601 ((*S*)-*N*-(2-chloro-3-(trifluoromethyl)benzyl)-1-(2-chloroethyl)-5-oxopyrrolidine-2-carboxamide, **3b**) were synthesized as outlined in Scheme 1, according to the published methods.[7] *tert*-Butyl (*S*)-5-oxopyrrolidine-2-carboxylate was reacted with

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* Corresponding author.
E-mail address: qzheng@iupui.edu (Q.-H. Zheng).

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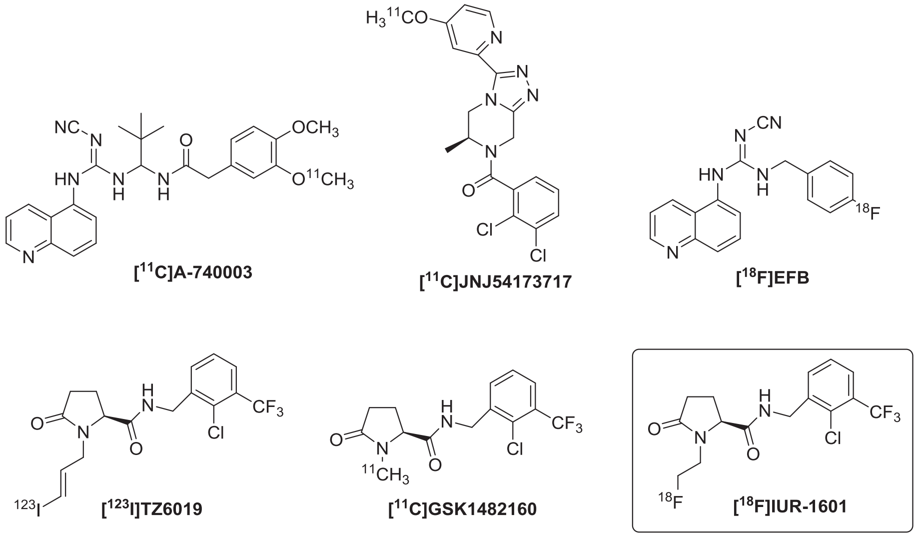
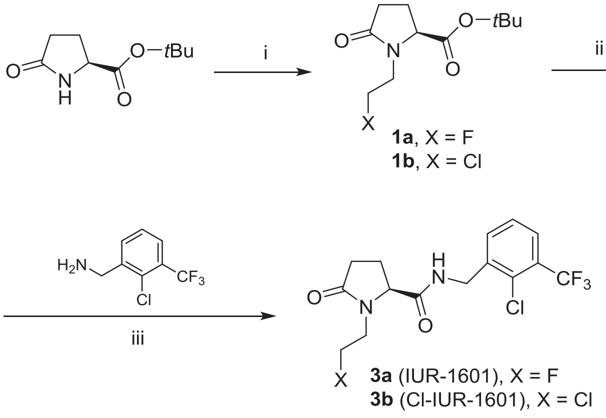


Fig. 1. Radioligands for imaging of P2X7R.



Scheme 1. Synthesis of IUR-1601 (**3a**) and its precursor Cl-IUR-1601 (**3b**). Reaction conditions and regents: (i) $BrCH_2CH_2X$, NaH, DMF; 42% and 38%. (ii) TFA, CH_2Cl_2. (iii) EDAC, HOBt, CH_2Cl_2, 28% and 27%.

1-bromo-2-fluoroethane (or 1-bromo-2-chloroethane) and NaH in *N,N*-dimethylformamide (DMF) to convert into amide *N*-alkylated product **1a** (or **1b**) in 42% and 38% yield, respectively. Subsequently, compound **1a** (or **1b**) was treated with trifluoroacetic acid (TFA) to give acid **2a** (or **2b**). Without further purification, compound **2a** (or **2b**) was undergone a coupling reaction with 2-chloro-3-(trifluoromethyl)benzylamine using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDAC) or 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) as catalyst, affording IUR-1601 (or Cl-IUR-1601) in about 28% and 27% yield, respectively.

Another precursor TsO-IUR-1601 ((*S*)-2-(2-((2-chloro-3-(trifluoromethyl)benzyl)carbamoyl)-5-oxopyrrolidin-1-yl)ethyl 4-methylbenzenesulfonate) was obtained via a customer synthesis service.

One-step radiosynthesis of [^{18}F]IUR-1601 was performed as indicated in Scheme 2 via a published method using K[^{18}F]F/Kryptofix2.2.2 and dimethyl sulfoxide (DMSO) as solvent at 140 °C for [^{18}F]fallypride production.[11] Both Cl-IUR-1601 and TsO-IUR-1601 precursors failed to produce the radiolabeled product [^{18}F]IUR-1601. At the beginning, we suspected the reaction temperature was too high, then we conducted a model fluorination reaction without radioactivity at 60 °C, which was monitored by analytical reverse-phase (RP) high performance liquid chromatography (HPLC). No IUR-1601 was detected, instead a compound (*S*)-*N*-(2-chloro-3-(trifluoromethyl)benzyl)-5-oxo-1-vinylpyrrolidine-2-carboxamide (vinyl-IUR-1601) was formed and detected by analytical RP HPLC, and confirmed by liquid chromatography-mass spectra (LC-MS), MS (ESI): $[M+H]^+$ 347. The result was summarized in Scheme 3.

A few other model reactions were carried out as shown in Schemes 4 and 5. The results suggested that TsO-IUR-1601 and Cl-IUR-1601 precursors are unstable, and they would easily convert to vinyl-IUR-1601 via elimination reaction at room temperature (RT); IUR-1601 is also unstable under strong base and high temperature conditions, and it would partially form vinyl-IUR-1601 via elimination reaction as well; and thus we concluded it is impossible to use one-step radiosynthesis starting from either tosyl ethyl- or chloro ethyl-precursor to prepare [^{18}F]IUR-1601.

Therefore, we turned our effort to two-step radiosynthesis of [^{18}F]IUR-1601 using desmethyl-GSK1482160 precursor and radiolabeled precursor 2-[^{18}F]fluoroethyl tosylate (2-[^{18}F]FCH_2CH_2OTS)[12] prepared from 1,2-ethylene glycol-bis-tosylate ($TsOCH_2CH_2OTs$) and K[^{18}F]F/Kryptofix2.2.2. Prior to the radiosynthesis, several model reactions in small scale (0.5–1.0 mg) without radioactivity were performed to investigate the solid base and reaction temperature effect on the fluoroethylation of cyclic amide of the desmethyl-GSK1482160 precursor, as