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Synthesis of *N*-(3-(4-[^{11}C]methylpiperazin-1-yl) − 1-(5-methylpyridin-2-yl) − 1*H*-pyrazol-5-yl)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide as a new potential PET agent for imaging of IRAK4 enzyme in neuroinflammation

Xiaohong Wang[a], Wenzhi Xu[a], Caihong Miao[a], Fugui Dong[a], Wei Li[a], Min Wang[b], Mingzhang Gao[b], Qi-Huang Zheng[b,*], Zhidong Xu[a,*]

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

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

HIGHLIGHTS

- A new carbon-11-labeled amidopyrazole inhibitor of IRAK4 was synthesized.
- A fully automated multi-purpose [^{11}C]-radiosynthesis module was built up.
- A semi-preparative RP HPLC-SPE technique was employed in radiosynthesis.

ARTICLE INFO

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Interleukin-1 receptor-associated kinase 4 (IRAK4)
Radiosynthesis
Positron emission tomography (PET)
Neuroinflammation

ABSTRACT

The reference standard *N*-(3-(4-methylpiperazin-1-yl) − 1-(5-methylpyridin-2-yl) − 1*H*-pyrazol-5-yl)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide (**9**) and its demethylated precursor *N*-(1-(5-methylpyridin-2-yl) − 3-(piperazin-1-yl) − 1*H*-pyrazol-5-yl)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide (**8**) were synthesized from pyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid and ethyl 2-cyanoacetate with overall chemical yield 13% in nine steps and 14% in eight steps, respectively. The target tracer *N*-(3-(4-[^{11}C]methylpiperazin-1-yl) − 1-(5-methylpyridin-2-yl) − 1*H*-pyrazol-5-yl)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide ([^{11}C]**9**) was prepared from its precursor with [^{11}C]CH_3OTf through *N*-[^{11}C]methylation and isolated by HPLC combined with SPE in 50–60% radiochemical yield, based on [^{11}C]CO_2 and decay corrected to EOB. The radiochemical purity was > 99%, and the specific activity at EOB was 370–1110 GBq/μmol.

1. Introduction

Inflammation is a complex biological process and part of the body's immune response involving immune cells, blood vessels, and molecular mediators for self-protection to remove harmful stimuli, including damaged cells, irritants, or pathogens (Rodero and Crow, 2016). Neuroinflammation is the inflammation of the nervous tissue, and it is associated with central nervous system (CNS) diseases like Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), traumatic brain injury (TBI) and stroke (Chen et al., 2016; Knezevic and Mizrahi, 2018; Rodero and Crow, 2016; Tronel et al., 2017). Molecular imaging of neuroinflammation in neurodegenerative diseases by positron emission tomography (PET) is one of the most active as well as most challenging areas in neuroscience, because PET neuroimaging can offer various non- or minimally invasive techniques to characterize neuroinflammatory processes for the purpose of diagnosis, therapy and treatment monitoring (Calsolaro and Edison, 2016; Cerami et al., 2017; Kielian, 2014; Schain and Kreisl, 2017). Many enzyme- or receptor-based radioligands have been developed for *in vivo* PET visualization of neuroinflammation (Albrecht et al., 2016; Gargiulo et al., 2017; Ory et al., 2014). We are interested in the development of new PET radioligands for neuroinflammation. In our previous work, we have synthesized and developed a series of PET radiotracers (Gao et al., 2010, 2011, 2015, 2017a; Territo et al., 2017; Wang et al., 2009; Zheng et al., 2003) that target the enzyme or receptor linked to neuroinflammation such as [^{11}C]FMAME for matrix metalloproteinase (MMP), carbon-11-labeled celecoxib derivatives for cyclooxygenase-2 (COX-2), [^{11}C]

* Corresponding authors.
E-mail addresses: qzheng@iupui.edu (Q.-H. Zheng), zhidongxu@hbu.edu.cn (Z. Xu).

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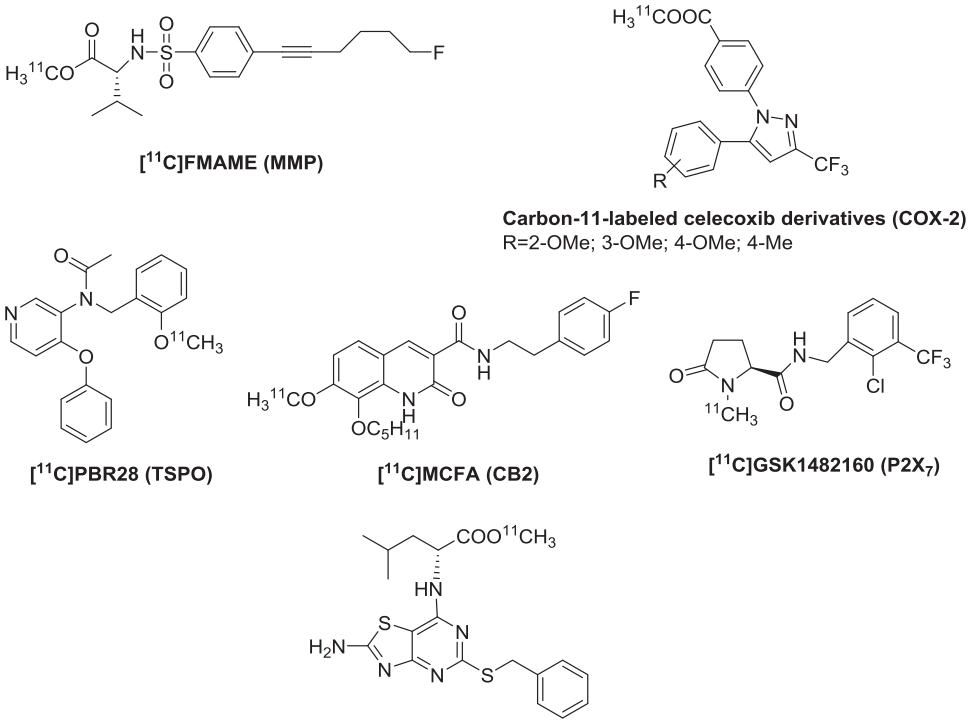

Fig. 1. PET radiotracers for imaging of neuroinflammation.

PBR28 for translocator protein (TSPO), [^{11}C]MCFA for cannabinoid receptor 2 (CB2), [^{11}C]GSK1482160 for purinergic receptor ($P2X_7$), and [^{11}C]methyl (2-amino-5-(benzylthio)thiazolo[4,5-*d*]pyrimidin-7-yl)-*D*-leucinate for CX_3C chemokine receptor 1 (CX_3CR1), as indicated in Fig. 1. These PET tracers may have different imaging mechanisms, unfortunately, they have been found to have some drawbacks as an "inflammation" radiotracer. For example, in humans TSPO ligand [^{11}C] PBR28 exhibited high inter-subject variability in binding affinity, with a genetic polymorphism of the TSPO target resulting in population stratification into high-, mixed- and low-affinity binders (Yoder et al., 2013). Thus, new "inflammation" PET tracers remain to be developed. In this ongoing study, we first select the enzyme interleukin-1 receptor-associated kinase 4 (IRAK4) as another more specific neuroinflammatory target for PET imaging. The enzyme IRAK4 represents a novel inflammation-associated molecular target. Radiotracers that target IRAK4 have the potential to overcome the limitations associated with previous "inflammation" radiotracers. IRAK4 is a critical upstream kinase in neuroinflammation and plays an important role in the progression of various neurodegenerative diseases (Jeong et al., 2017; Lv et al., 2017; Wang et al., 2014; Yuan et al., 2015). Recently, a potent and selective amidopyrazole inhibitor of IRAK4 with IC_{50} 5 nM, *N*-(3-(4-methylpiperazin-1-yl) − 1-(5-methylpyridin-2-yl) − 1*H*-pyrazol-5-yl) pyrazolo[1,5-*a*]pyrimidine-3-carboxamide (**9**), has been developed by Merck (McElroy et al., 2015). However, the PubMed search showed no records on radiolabeled IRAK4 inhibitors. Here we report the design and synthesis of a new carbon-11-labeled IRAK4 amidopyrazole inhibitor *N*-(3-(4-[^{11}C]methylpiperazin-1-yl) − 1-(5-methylpyridin-2-yl) − 1*H*-pyrazol-5-yl)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide ([^{11}C] **9**) as a candidate PET neuroinflammation imaging agent.

2. Results and discussion

2.1. Chemistry

The reference standard **9** and its demethylated precursor *N*-(1-(5-methylpyridin-2-yl) − 3-(piperazin-1-yl) − 1*H*-pyrazol-5-yl)pyrazolo [1,5-*a*]pyrimidine-3-carboxamide (**8**) were synthesized as depicted in Scheme 1, according to the published procedures (Gopalsamy et al., 2009; Lim and Altman, 2015; McElroy et al., 2015) with modifications. Pyrazolo[1,5-*a*] pyrimidine-3-carbonyl chloride (**1**) was achieved by the reaction of commercially available pyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid with thionly chloride. Compound **1** was used directly without further purification. 2-Cyano-3,3-bis(methylthio)acrylic acid (**3**) was prepared from ethyl 2-cyanoacetate by condensation with carbon disulfide in the presence of aqueous NaOH in EtOH, followed by hydrolysis with aqueous NaOH and methylation with dimethyl sulfate based on the reported procedure (Henriksen, 1996), with an overall chemical yield 54% for two steps. Commercially available *tert*-butyl piperazine-1-carboxylate and compound **3** underwent combined substitution and decarboxylation in the presence of trimethylamine (TEA) in MeOH to give (*Z*)-*tert*-butyl 4-(2-cyano-1-(methylthio)vinyl)piperazine-1-carboxylate (**4**) in 70% yield. Condensation of **4** with hydrazine monohydrate in EtOH afforded pyrazole derivative *tert*-butyl 4-(5-amino-1*H*-pyrazol-3-yl)piperazine-1-carboxylate (**5**) in 90% yield. Coupling of pyrazole derivative **5** and 2-bromo-5-methylpyridine employed CuI as catalyst, (1*S*,2*S*)-N^1,N^2-dimethylcyclohexane-1,2-diamine as organic ligand in the presence of Cs_2CO_3 in dimethyl sulfoxide (DMSO) to afforded *tert*-Butyl 4-(5-amino-1-(5-methylpyridin-2-yl) − 1*H*-pyrazol-3-yl)piperazine-1-carboxylate (**6**) in 60% yield. Amidation of acyl halide **1** with amine **6** in the presence of *N*, *N*-diisopropylethylamine (DIPEA) in CH_2Cl_2 gave amide derivative **7** in 73% yield, which was deprotected Boc group with trifluoroacetic acid (TFA) in CH_2Cl_2 to yield the precursor **8** in 95% yield. N-methylation was performed by reductive amination of compound **8** with formaldehyde by $NaBH(OAC)_3$ in CH_2Cl_2 to obtain the reference standard **9** in 98% yield. The specific modifications to the published synthetic procedures were major in the optimization of the reaction conditions in each step to improve the synthetic yield. For instance, we used TFA/CH_2Cl_2 instead of HCl/dioxane in the reported procedure (McElroy et al., 2015) for the deprotecting reaction of Boc group of compound **7** to give the precursor **8** in high yield.