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Research paper

Evaluation of the novel TSPO radiotracer 2-(7-butyl-2-(4-(2-([^{18}F]fluoroethoxy)phenyl)-5-methylpyrazolo[1,5-*a*]pyrimidin-3-yl)-*N*,*N*-diethylacetamide in a preclinical model of neuroinflammation

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ABSTRACT

Translocator Protein (18 kDa, TSPO) is regarded as a useful biomarker for neuroinflammation imaging. TSPO PET imaging could be used to understand the role of neuroinflammation in brain diseases and as a tool for evaluating novel therapeutic effects. As a promising TSPO probe, [^{18}F]DPA-714 is highly specific and offers reliable quantification of TSPO *in vivo*. In this study, we further radiosynthesized and evaluated another novel TSPO probe, 2-(7-butyl-2-(4-(2-[^{18}F]fluoroethoxy)phenyl)-5-methylpyrazolo[1,5-*a*]pyrimidin-3-yl)-*N*,*N*-diethylacetamide ([^{18}F]VUIIS1018A), which features a 700-fold higher binding affinity for TSPO than that of [^{18}F]DPA-714. We evaluated the performance of [^{18}F]VUIIS1018A using dynamic *in vivo* PET imaging, radiometabolite analysis, *in vitro* autoradiography assays, biodistribution analysis, and blocking assays. *In vivo* study using this probe demonstrated high signal-to-noise ratio, binding potential (BP_{ND}), and binding specificity in preclinical neuroinflammation studies. Taken together, these findings indicate that [^{18}F]VUIIS1018A may serve as a novel TSPO PET probe for neuroinflammation imaging.

1. Introduction

The central nervous system (CNS) will promote homeostasis by enacting a series of responses both in physiological and pathological states. These responses are referred to as "neuroinflammation" and encompass cellular and molecular actions to identify the damaged event and then to repair any consequent damage [1]. Neuroinflammatory responses can occur in many disease states, such as neurodegenerative diseases and autoimmune disorders [2–4]. Neuroinflammation often occurs in the presence of a local insult or the distally existing pathological events that can disturb the normal functioning in the CNS [5]. As neuroinflammation has relationship with various neuropathologies, many studies for diagnosis and therapy monitoring of neuroinflammation, and guiding the development of novel therapies have been performed for the past 15 years [5]. Among those studies, neuroimaging provides a non-invasive tool to characterize and monitor the neuroinflammation *in vivo*. As one of the neuroimaging modalities, nuclear medicine imaging using positron emission tomography (PET) can be employed to visualize and quantify the molecular target at a nanomolar level, with high sensitivity and specificity.

As a useful imaging biomarker for neuroinflammation,

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translocator protein (18 kDa, TSPO) has been studied for over 20 years [6]. Formally referred to as the peripheral benzodiazepine receptor, TSPO is a membrane protein located primarily on the outer mitochondrial membrane [7], and it is widely located in many peripheral organs including the lungs, heart, kidneys, and nasal epithelium [8]. In the normal brain, TSPO is expressed in multiple cell types at very low levels [8], and it will be overexpressed when microglia are activated in response to an injury in neuroinflammation [5]. Thus, the expression of TSPO has been considered as a biomarker for neuroinflammation in numerous neurological diseases including Alzheimer's diseases (AD), Parkinson's disease (PD), multiple sclerosis (MS), Huntington's disease (HD), and amyotrophic lateral sclerosis (ALS) [9]. In previous studies, TSPO imaging using PET has been employed as a useful tool for *in vivo* imaging and evaluation of neuroinflammation in clinic [5]. In both the clinical and preclinical studies, TSPO imaging has been used to understand the roles of neuroinflammation and assesse the novel therapeutic effects for brain diseases.

Radiotracers for TSPO PET imaging have been used in neuroinflammation for over 20 years [5]. The most common radiotracer used to date is [^{11}C]PK11195 [6], which nonetheless has several limitations, including low brain penetrance, high non-specific binding, low sensitivity, low signal-to-noise ratio, and poor amenability to quantification [10]. Consequently, many studies have been conducted to develop novel TSPO radiotracers, including phenoxyarylacetamides derivatives labeled with ^{11}C ([^{11}C]DAA1106, [^{11}C]PBR28) or ^{18}F ([^{18}F]FEDAA1106, [^{18}F]FEPPA, [^{18}F]PBR06), imidazopyridines derivatives ([^{11}C]CLINME), and pyrazolopyrimidines derivatives ([^{18}F]DPA-714) [11]. Among these, [^{18}F]DPA-714 is a pyrazolopyrimidinal probe featuring a high binding affinity to TSPO [12,13]. Labeled with ^{18}F, [^{18}F]DPA-714 has a longer half-time (110 min) than [^{11}C]PK11195 (20 min). It is used widely in a novel modality for dynamically monitoring the brain inflammatory response and disease progression, and showed improved bioavailability, lower nonspecific binding, and higher non-displaceable binding potential (BP_{ND}) [14]. Clinical studies using [^{18}F]DPA-714 have been performed on patients with ALS [15,16] and AD [16,17], as well as in post-stroke studies [18], and showed great promise in neuroimaging studies. Based on the scaffold of DPA-714, a variety of novel probes has been developed and used in TSPO PET imaging, including [^{18}F]DPA-C5yne [19], [^{18}F]VUIIS1008 [20,21], [^{18}F]VUIIS1009A/B [22], and [^{18}F]FDPA [23]. All those probes showed promising results in the *in vivo* dynamic PET imaging.

In this study, we synthesized and evaluated another novel pyrazolopyrimidinal TSPO PET probe, 2-(7-butyl-2-(4-(2-[^{18}F]fluoroethoxy)phenyl)-5-methylpyrazolo[1,5-*a*]pyrimidin-3-yl)-*N*,*N*-diethylacetamide ([^{18}F]VUIIS1018A, Fig. 1), for neuroinflammation in a preclinical cerebral ischemia model. VUIIS1018A (IC_{50}: 16.2 pM) features a 700-fold higher *in vitro* TSPO binding affinity than DPA-714 (IC_{50}: 10.9 nM) [24] and we further evaluated the performance of this probe using dynamic *in vivo* PET imaging, radiometabolite analysis, *in vitro* autoradiography assays, biodistribution analysis, and blocking assays.

2. Results

2.1. Radiosynthesis

By [^{18}F]fluoroalkylation of the phenol precursor with [^{18}F]fluoroethyl bromide ([^{18}F]FEtBr), [^{18}F]VUIIS1018A with 1.4–2.1 GBq was obtained as an injectable solution at the end of synthesis (EOS), starting from 7.4 GBq of cyclotron-produced [^{18}F]F^- (Scheme 1). The decay-corrected radiochemical yield was 33 ± 9% ($n = 7$) based on [^{18}F]F^- in the synthesis times of 63 ± 2 min from end of bombardment, and the molar activity was 120–190 GBq/μmol at EOS. This product had a radiochemical purity >95% and did not show radiolysis at room temperature within 90 min after radiosynthesis, indicating its radiochemical stability for the duration of at least one PET scan.

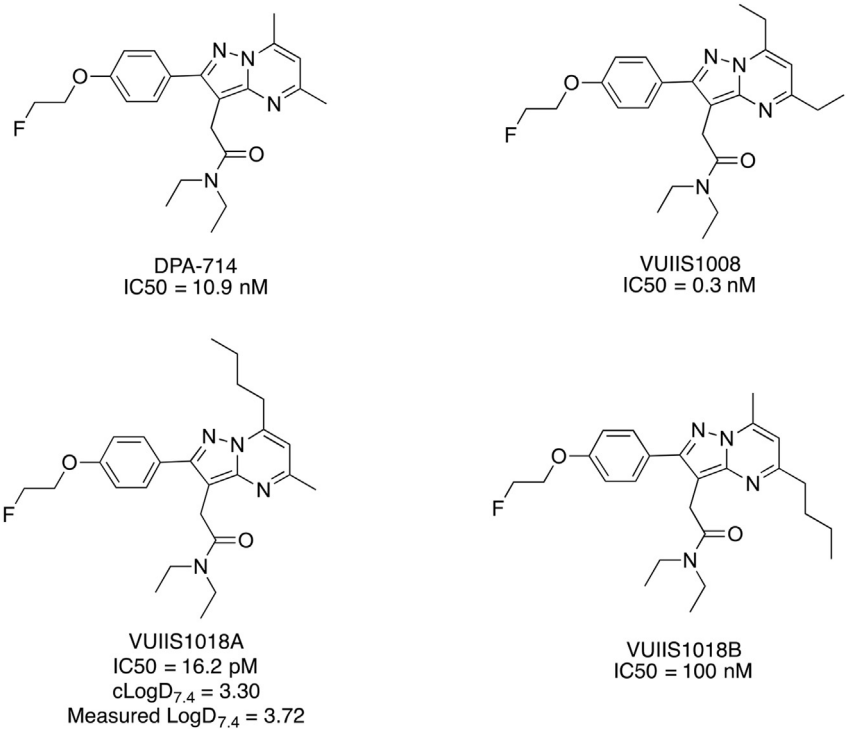

Fig. 1. DPA-714 and its derivatives.