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Synthesis and biological evaluation of novel ^{123}I-labeled 4-(4-iodophenyl)butanoyl-L-prolyl-(2*S*)-pyrrolidines for imaging prolyl oligopeptidase *in vivo*

Annukka Kallinen [a,*], Boyan Todorov [a], Roope Kallionpää [b], Susanne Bäck [c], Mirkka Sarparanta [a], Mari Raki [d], Juan A. García-Horsman [c], Kim A. Bergström [d,e], Erik A.A. Wallén [b], Pekka T. Männistö [c], Anu J. Airaksinen [a,*]

[a] Laboratory of Radiochemistry, Department of Chemistry, P.O. Box 55, FI-00014 University of Helsinki, Finland
[b] Division of Pharmaceutical Chemistry, Faculty of Pharmacy, P.O. Box 56, 00014 University of Helsinki, Finland
[c] Division of Pharmacology and Toxicology, Faculty of Pharmacy, P.O. Box 56, 00014 University of Helsinki, Finland
[d] Centre for Drug Research, Faculty of Pharmacy, P.O. Box 56, 00014 University of Helsinki, Finland
[e] HUS Medical Imaging Center, Helsinki University Central Hospital, Finland

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ABSTRACT

Prolyl oligopeptidase (POP) may be associated with neuromodulation and development of neurodegenerative diseases and it was recently shown to participate in the inflammatory cascade along with matrix metalloproteinases. Radiotracers, which can be used for non-invasive imaging, are needed for investigating the role of POP in normal physiology and in pathophysiological conditions *in vivo*. We synthesized two novel POP-specific ^{123}I-radiolabeled 4-phenylbutanoyl-L-prolyl-pyrrolidines of which 4-(4-[^{123}I]iodophenyl)butanoyl-L-prolyl-2(*S*)-cyanopyrrolidine ([^{123}I]**2f**, K_i = 4.2 nM) was selected. The selected compound has an electrophilic cyano group that is known to increase the dissociation time of POP inhibitors. [^{123}I]**2f** was synthesized in high radiochemical yield and purity (87 ± 4%, >99%, respectively) and with a specific activity of 456 ± 98 GBq/μmol. [^{123}I]**2f** was evaluated in healthy mice (C57Bl/6JRccHsd) by *ex vivo* biodistribution studies and SPECT imaging. Pretreatment with the known inhibitor 4-phenylbutanoyl-L-prolyl-(*2S*)-cyanopyrrolidine (KYP-2047, **2d**, K_i = 0.023 nM) showed that binding of [^{123}I]**2f** was POP specific. In addition, [^{123}I]**2f** was evaluated in models of neuroinflammation and acute localized inflammation. A minor increase in binding of [^{123}I]**2f** was observed in the inflamed region in the acute localized inflammation model. Similar increase in binding was not observed in the neuroinflammation model.

1. Introduction

Prolyl oligopeptidase (POP, also known as prolyl endopeptidase, PREP) (EC 3.4.21.26) is an endopeptidase that hydrolyzes L-proline peptide bonds on the carboxylic acid side of L-proline residue in peptides comprised of a maximum of 30 amino acids. It belongs to a larger prolyl oligopeptidase family S9 which includes enzymes dipeptidyl peptidase IV (DPPIV), acylaminoacyl peptidase and oligopeptidase B [1,2]. Most of the studies indicate that POP is a soluble cytosolic enzyme, although evidence for the existence of a membrane bound form of the enzyme has also been presented [3]. POP has been suggested to have a role in the metabolism of neuropeptides. However, studies with POP inhibitors have ended up with contradicting results, maintaining the role of the enzyme in the metabolism of the neuropeptides elusive. [4,5] Alternatively, POP has been shown to be involved in the cycle of inositol triphosphate (IP_3), a secondary messenger transmitting the receptor-mediated signaling of several neuropeptides [6]. Lately, POP activity has been correlated to an increase in the accumulation of α-synuclein, an insoluble fibrillar inclusion typically found in Parkinson's disease (PD) and in Lewy body dementia [7,8]. Moreover, it was shown very recently that the inhibition of POP resulted in a decrease of α-synuclein accumulation in an α-synuclein-expressing transgenic mouse model [9], and that POP co-localizes

Abbreviations: POP, Prolyl oligopeptidase; PD, Parkinson's disease; MMP, matrix metalloproteinases; LPS, lipopolysaccharide.

* Corresponding authors.
E-mail addresses: annukka.kallinen@helsinki.fi, annukka.kallinen@ansto.gov.au (A. Kallinen), anu.airaksinen@helsinki.fi (A.J. Airaksinen).

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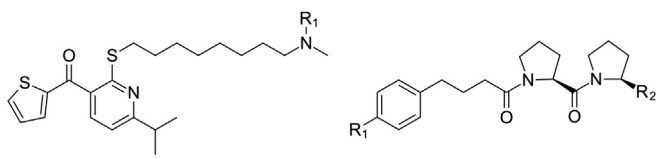
with α-synuclein in the brain of PD patients [10]. In the same study it was also shown that POP co-localizes with the tau protein.

Recently, several studies have indicated that POP may have a role in inflammatory diseases [11]. POP has been found in granule-like structures in the cytoplasm of neutrophils [12]. Other studies implicate concerted action of POP and matrix metalloproteinases (MMP) in the degradation of collagen at the site of inflammation. The MMPs degrade the collagen strands thus exposing prolyl-glycyl-proline (PGP) moieties that serve as substrates for POP. The tripeptide PGP, specifically cleaved and released by POP, can subsequently act as a chemoattractant for neutrophils [11]. In multiple sclerosis, an autoimmune disease where matrix metalloproteinases are upregulated, POP activity has been found consistently altered in the forms of the disease with a strong inflammatory component [13]. These findings suggest a rationale for studying the possible involvement of POP in neurodegenerative diseases where an inflammatory component is typically found, and support its possible role as a disease biomarker [14].

In vivo molecular imaging is an important tool in investigation of biological processes and molecular interactions in preclinical animal models. A synthesis and use of a tight-binding fluorescent POP inhibitor for the detection of POP in cell cultures via optical imaging has been reported earlier [15,16]. By using imaging modalities based on distribution of radiolabeled tracer molecules, such as positron emission tomography (PET) and single photon emission computed tomography (SPECT), the preclinical imaging methodology can be readily translated to clinical applications. However, investigation of the role of POP in normal physiology and in pathophysiological conditions, especially in the CNS, has been hampered by the lack of an imaging tracer specific for POP. By developing radiolabeled POP inhibitors for *in vivo* imaging, new information on the localization of the enzyme and its role in neuromodulation and development of neurodegenerative and inflammatory diseases could be obtained. To the best of our knowledge, only one research group has published radiolabeled POP inhibitors. The syntheses of radiolabeled POP inhibitors ((2-(8-(*N*,*N*-([^{11}C]methyl)methylamino)octylthio))-6-isopropyl)-3-pyridyl 2-thienyl ketone ([^{11}C]**1b**, K_i = 0.95 nM, rat brain POP) [17] and *N*-((*N*-(-4-(*N*-[^{11}C]methylamino)phenyl)butyryl)-L-prolyl)pyrrolidine ([^{11}C]**2c**, IC_{50} = 3.1 nM, mouse brain POP) [18] from the corresponding desmethylated precursors **1a** and **2b** (Fig. 1) via ^{11}C-methylation have been described previously, although no biological evaluation of these tracers has been reported [19,20].

To add a new tool for the research on the physiological role of POP, and in search of new diagnostic applications, we decided to synthesize ^{123}I-labeled analogues of **2a** (SUAM-1221) [21] and **2d** (KYP-2047) [22] (Fig. 1) and to investigate the biodistribution in healthy mice with SPECT/CT. The K_i values for **2a** and **2d** are 0.97 nM and 0.023 nM, respectively [23]. Inhibitor **2d** is a slow, tight-binding inhibitor [23], because it forms an imino ether adduct with the enzyme [24]. The long-lasting inhibition would provide a possibility for the signal detection over several hours [23]. The *para*-position of the phenyl ring was chosen as the site for introducing the ^{123}I-label, as different substituents have previously been added to this position without substantial changes in the binding affinity. The aromatic position should also result in a relatively stable radiolabeling, when considering the *in vivo* stability of the final structure.



1a (desmethyl Y-29794): R_1 = H
[^{11}C]**1b**: R_1 = $^{11}CH_3$

2a (SUAM-1221): R_1 = H; R_2 = H
2b: R_1 = NH_2; R_2 = H
[^{11}C]**2c**: R_1 = NH[^{11}C]CH_3, R_2 = H
2d (KYP-2047): R_1 = H; R_2 = CN
2e: R_1 = I; R_2 = H
2f: R_1 = I; R_2 = CN

Fig. 1. Structures of POP inhibitors **1a** (desmethyl Y-29794) and **2a** (SUAM-1221) and their radiolabeled derivatives.

In this study we have described the synthesis, radiosynthesis and *in vitro* characterization of the ^{123}I-labeled analogues of POP inhibitors **2a** and **2d**. We have also presented the first evaluation of a radiolabeled POP inhibitor *in vivo* in mice. Considering the possible involvement of POP in inflammation and its potential use as an imaging biomarker for inflammation, we made a preliminary evaluation of the selected radiolabeled POP inhibitor in two animal models of acute inflammation.

2. Results & discussion

2.1. Chemistry

The iodide substituted analogues **2e** and **2f** of the known POP inhibitors **2a** (SUAM-1221) and **2d** (KYP-2047), respectively, were synthesized in good yields following a general synthetic procedure originally reported by Arai et al. (Scheme 1) [22,25].

2.2. Evaluation of the binding properties with in vitro enzyme assay

To determine whether the *p*-iodo-substituent on the phenyl ring affected the inhibitory activity, the apparent K_i values of **2d**, **2e** and **2f** were determined. The obtained values for **2d**, **2e** and **2f** were 0.1, 22 and 4.2 nM, respectively (Fig. 2). These results indicate that the *p*-iodination decreased the inhibitory activity of the compounds, however the constants still are at low nanomolar range. Because of the higher inhibitory potency over **2e**, **2f** was chosen for further *in vitro* characterization and *in vivo* studies.

2.3. Radiochemistry

For the radiosynthesis, **2e** and **2f** were treated with hexamethylditin in the presence of $Pd(PPh_3)_4$-catalyst to give the stannane precursors **5a** and **5b** in 73% and 75% yield, respectively (Scheme 2). The radiosynthetic step was performed according to the methods described in the literature [26]. First, the oxidation was optimized with respect to radiochemical yield and the purity by testing different oxidation agents. The relatively mild oxidant Iodogen® was tested for precursor **5b** resulting in a low radiochemical yield of [^{123}I]**2f** (15%) (Table 1). Chloramine-T (CAT) gave moderate to high radiochemical yields (80–90%), however, an unwanted UV-active side product eluting simultaneously to the tracer was detected in the HPLC purification. The side product may have resulted from the chlorination side reactions often encountered with CAT based oxidants. Lastly, peracetic acid was tested, which resulted in high radiochemical yields (>99%) and purities (>99%) of [^{123}I]**2e** and [^{123}I]**2f** in a small-scale synthesis (radioactivity 10 MBq, 1.14 pmol of ^{123}I). The optimal reaction conditions proved to be the reaction time of 10 min at room temperature in aqueous solution containing 20 equiv of peracetic acid in neutral conditions, and the use of 12 equiv of metabisulphite to quench the oxidant. On the basis of *in vitro* enzyme assay studies, it was decided to continue to *in vivo* evaluation with tracer [^{123}I]**2f** showing a higher affinity to the target enzyme. The optimized reaction conditions were used in a larger scale synthesis with the starting activities ranging from 300 to 680 MBq. [^{123}I]**2f** was obtained with an average radiochemical