Applied Radiation and Isotopes 74 (2013) 61–69

Contents lists available at SciVerse ScienceDirect

Applied Radiation and Isotopes

journal homepage: www.elsevier.com/locate/apradiso

Synthesis of radiolabeled protein disulfide isomerase (PDI) inhibitors as new potential PET agents for imaging of the enzyme PDI in neurological disorders and cancer

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HIGHLIGHTS

- New substituted β-tetrahydrocarbolines were synthesized.
- New radiolabeled substituted β-tetrahydrocarbolines were synthesized.
- Simple solid-phase extraction (SPE) method was employed in radiosynthesis.

ARTICLE INFO

Article history:
Received 13 October 2012
Received in revised form
29 November 2012
Accepted 1 January 2013
Available online 8 January 2013

Keywords:
Radiolabeled substituted
β-tetrahydrocarbolines
Protein disulfide isomerase (PDI)
Positron emission tomography (PET)
Radiosynthesis
Neurological disorders
Cancer

ABSTRACT

Carbon-11-labeled substituted β-tetrahydrocarbolines were prepared from their corresponding phenolic hydroxyl precursors with [^{11}C]CH_3OTf through *O*-[^{11}C]methylation and isolated by simplified SPE in 50–60% decay corrected radiochemical yields at EOB with 185–370 GBq/μmol specific activity at EOS. A fluorine-18-labeled substituted β-tetrahydrocarboline was prepared from its corresponding halo-precursors (X=Cl, Br, I) with K[^{18}F]F/Kryptofix 2.2.2 via the nucleophilic substitution and isolated by HPLC combined with SPE in 25–40% decay corrected radiochemical yield with 37–222 GBq/μmol specific activity at EOB.

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1. Introduction

Protein disulfide isomerase (PDI) is an enzyme in the endoplasmic reticulum (ER) of eukaryotic cells (Wilkinson and Gilbert, 2004). PDI is primarily involved in protein folding by oxidation or isomerization and disrupts disulfide bonds by reduction (Khan et al., 2011). PDI also has essential role in chaperone-mediated quality control of proteins in the secretory pathway (Benham, 2012). In addition, PDI is linked to cell survival and apoptotic cell death (apoptosis) (Hoffstrom et al., 2010; Laurindo et al., 2012). Misfolded proteins, leading to cellular dysfunction and cell death, accumulate in a number of neurological disorders such as Alzheimer's disease, Parkinson's disease, Huntington's disease and amyotrophic lateral sclerosis (ALS), and cancers (Laurindo et al., 2012). PDI is considered as an interesting potential drug target, and discovering function-specific inhibitors for this enzyme is a significant challenge in the therapeutic targeting of PDI (Khan et al., 2011). A wide range of therapeutic agents like antibiotics, thiol blockers, estrogenic compounds and arsenical compounds have been screened for this purpose. However, only few really specific inhibitors were discovered (Khan et al., 2011). Recently a novel series of small-molecule PDI inhibitors, substituted β-tetrahydrocarbolines, have been developed, which suppress apoptosis induced by misfolded proteins and show significant potential to aid the discovery of therapeutics to treat protein-misfolding diseases (Hoffstrom et al., 2010). These β-tetrahydrocarboline compounds were derived from the strategies for the synthesis of novel indole alkaloid-based screening

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0969-8043/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.apradiso.2013.01.003

libraries for drug discovery and chemical biology study (Fokas et al., 2005). PDI has attracted our interest, because it is considered to be a target for biomarker for diagnostic *in vivo* imaging agent. The potential of PDI as a feasible target for molecular imaging has been explored by using different imaging modalities such as coherent X-ray diffraction microscopy, fluorescence microscopy (optical imaging), and two-dimensional gel electrophoresis (2D PAGE) (Gromov et al., 2010; Kodama and Nakasako, 2011; Popescu et al., 2012). The major limitation is the lack of techniques to assess non-invasively *in vivo* PDI expression in living cells, animals, and humans. Advanced molecular imaging techniques, like positron emission tomography (PET), may provide useful tools for detecting PDI enzyme levels in protein folding diseases and monitoring their response to PDI inhibitor treatment, since only PET has sufficient sensitivity and quantitation to measure the expression of enzymes *in vivo* (Ray et al., 2003). However, so far no specific and selective PDI PET imaging agent to guide therapy is developed and reported. We are interested in the development of PDI-based biomarkers. Here we report the design and synthesis of radiolabeled PDI inhibitors as new potential imaging agents for biomedical imaging technique PET to image the enzyme PDI in neurological disorders and cancer, for the first time.

2. Results and discussion

2.1. Chemistry

A series of PDI inhibitors, substituted β-tetrahydrocarbolines, were designed and synthesized from three different diversity sites of a 1,1,2-trisubstituted β-tetrahydrocarboline scaffold based on the literatures (Fokas et al., 2005; Hénin et al., 1999; Hoffstrom et al., 2010; Hudlicky et al., 1981), as indicated in Fig. 1. The ester group in the parent compound could be another diversity site as well. However, additional structure–activity studies have revealed that structural modifications of the ester from methyl to other groups did not affect its activity or resulted in a complete loss of activity (Hoffstrom et al., 2010).

As outlined in Scheme 1, β-tetrahydrocarbolines with general structure (**3a**–**f**) were prepared by a Pictet–Spengler reaction between trytamine and α-ketoester. Treatment of tryptamine hydrochloride **1** with α-ketoester **2** in MeOH at reflux provided the β-tetrahydrocarboline **3** in 65–85% yield. If free tryptamine is replaced, then HCl (6 N, 1.2 equiv.) should be added to the reaction. Acylation of **3** with halogen acid chloride using diisopropylethylamine as a catalyst gave halogen *N*-acylated β-tetrahydrocarboline **4a**–**r** in 50–72% yield, which would be the precursor or reference standard for radiolabeling.

Carbon-11 labeling could be placed at either methoxy position or methyl ester position of substituted β-tetrahydrocarbolines

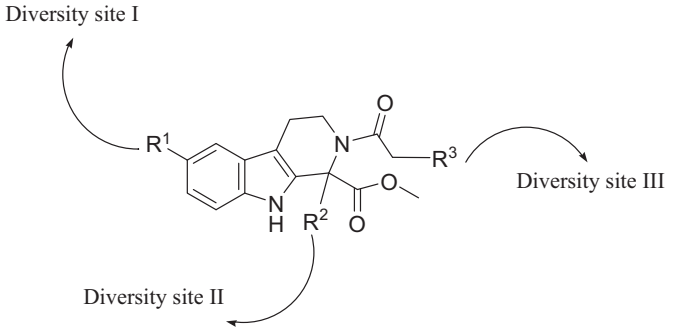

Fig. 1. Three diversity sites of a 1,1,2-trisubstituted β-tetrahydrocarboline scaffold.

through *O*-[^{11}C]methylation. As shown in Scheme 2, attempt to obtain the acid of **4g** via ester hydrolysis in MeOH as solvent and KOH as a base was failed, instead it gave compound **5** in 86% yield. Under the strong base condition, N-acylated chloride is more active than the methyl ester group, and an ether product **5** was formed. When boron tribromide was used for the cleavage of methyl ester, it afforded the acid of **4g**, but the acid of **4g** was not stable on silica gel during the flash column purification. Therefore, we were unable to obtain pure acid of **4g** for carbon-11 labeling at methyl ester position.

Using the similar method (Wang et al., 2011), the target compound fluoro-standard **4a** was prepared from its corresponding chloro-, bromo- and iodo-precursors **4g**, **m** and **p** through the nucleophilic substitution in 24–52% yield as indicated in Scheme 3. Chemical yield of **4a** from different halo-precursors was I-precursor (**4p**) > Br-precursor (**4m**) > Cl-precursor (**4g**). These halogen N-acylated β-tetrahydrocarbolines could be the precursors and authentic standard for fluorine-18 labeling.

2.2. Radiochemistry

Synthesis of carbon-11-labeled substituted β-tetrahydrocarbolines ([^{11}C]**4f**, [^{11}C]**4l**) is indicated in Scheme 4. Phenolic hydroxyl precursors (**4e**, **k**) were labeled by a reactive [^{11}C]methylating agent, [^{11}C]methyl triflate ([^{11}C]CH_3OTf) (Jewett, 1992; Mock et al., 1999) prepared from [^{11}C]CO_2, under basic conditions (2 N NaOH) in acetonitrile through the *O*-[^{11}C]methylation and isolated by a simplified solid-phase extraction (SPE) method (Gao et al., 2008, 2009, 2011, 2012) to provide corresponding target tracers [^{11}C]**4f**, [^{11}C]**4l** in 50–60% (n=5) radiochemical yields, decay corrected to end of bombardment (EOB), based on [^{11}C]CO_2. The large polarity difference between the phenolic hydroxyl precursor and the corresponding labeled *O*-methylated ether product permitted the use of SPE technique for purification of the labeled product from the radiolabeling reaction mixture. A C-18 Plus Sep-Pak cartridge was used in SPE purification technique. The crude reaction mixture was treated with aqueous $NaHCO_3$ and loaded onto the cartridge by gas pressure. The pH of freshly prepared 0.1 M $NaHCO_3$ might be too low to effectively deprotonate a phenol. However, an excess of 2 N NaOH used in the reaction provided a final pH after addition of the 0.1 M $NaHCO_3$ high enough to deprotonate all the phenol. Any non-reacted phenolic hydroxyl precursor was actually converted to the corresponding sodium salt, and any non-reacted [^{11}C]CH_3OTf was actually hydrolyzed to [^{11}C]CH_3OH, which would not be trapped to the C-18 Sep-Pak. The cartridge was washed with water to remove non-reacted [^{11}C]CH_3OTf, remaining phenolic hydroxyl precursor and reaction solvent, and total 6 mL (2 × 3 mL) volume of water was enough to wash off all phenol. The final labeled product was eluted with ethanol (2 × 2 mL), concentrated by rotary evaporation and reformulated in saline (10 mL). In our fully automated radiosynthesis module (Mock et al., 2005b; Wang et al., 2012b), it is difficult to directly elute the labeled product from a C-18 Sep-Pak to a vial using either 1 × 1 mL ethanol or 2 × 0.5 mL ethanol, due to the back pressure in the C-18 Sep-Pak and dead volume in the transfer tubing. In order to elute most of the labeled product from the C-18 Sep-Pak, we need to increase the volume of the eluent ethanol. For the radiotracers produced for animal study, we used 2 × 2 mL ethanol for elution, and evaporation was required before reformulation. For the radiotracer produced for human study, we used 2 × 1 mL ethanol, no evaporation required, and a C-18 Sep-Pak was used for direct reformulation (Gao et al., 2010; Wang et al., 2011, 2012a, 2012b). Overall synthesis time was 23 min from EOB, including approximately 11 min for [^{11}C]CH_3OTf production, 5 min for *O*-[^{11}C]methylation reaction, and 7 min for SPE purification, evaporation and reformulation. SPE technique is

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