



## Synthesis of [ $^{125}$ I]iodoDPA-713: A new probe for imaging inflammation

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### ARTICLE INFO

#### Article history:

Received 17 August 2009

Available online 22 August 2009

#### Keywords:

Pyrazolopyrimidine

Peripheral benzodiazepine receptor

Translocator protein

Autoradiography

Small animal imaging

### ABSTRACT

[ $^{125}$ I]iodoDPA-713 [ $^{125}$ I]**1**, which targets the translocator protein (TSPO, 18 kDa), was synthesized in seven steps from methyl-4-methoxybenzoate as a tool for quantification of inflammation in preclinical models. Preliminary *in vitro* autoradiography and *in vivo* small animal imaging were performed using [ $^{125}$ I]**1** in a neurotoxicant-treated rat and in a murine model of lung inflammation, respectively. The radiochemical yield of [ $^{125}$ I]**1** was  $44 \pm 6\%$  with a specific radioactivity of 51.8 GBq/ $\mu$ mol (1400 mCi/ $\mu$ mol) and >99% radiochemical purity. Preliminary studies showed that [ $^{125}$ I]**1** demonstrated increased specific binding to TSPO in a neurotoxicant-treated rat and increased radiopharmaceutical uptake in the lungs of an experimental inflammation model of lung inflammation. Compound [ $^{125}$ I]**1** is a new, convenient probe for preclinical studies of TSPO activity.

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### Introduction

Inflammation is becoming an increasingly important target for imaging, particularly in the context of central nervous system disease [1,2]. Imaging agents are also being sought to study autoimmune disease and the inflammatory arthritides, the cardiovascular system and cancer. For nearly 20 years the standard radiopharmaceutical for imaging inflammation has been the isoquinoline [ $^{11}$ C]PK11195, a ligand for the translocator protein (TSPO, 18 kDa), a.k.a. the peripheral benzodiazepine receptor (PBR), which is upregulated in activated glial and immune cells [3,4]. But [ $^{11}$ C]PK11195 tends to demonstrate significant non-specific binding and poor brain uptake [5,6], so superior radioligands for TSPO have been aggressively sought [2]. One such class of compounds that could be derivatized for imaging TSPO includes the pyrazolopyrimidines, in particular *N,N*-diethyl-2-[2-(4-methoxy-phenyl)-5,7-dimethyl-pyrazolo[1,5-*a*]pyrimidin-3-yl]-acetamide, DPA-713 [7,8]. DPA-713 is 10-fold less lipophilic than PK11195 and has nearly twice the affinity for TSPO. James et al. synthesized [ $^{11}$ C]DPA-713, which demonstrated higher signal-to-noise ratios than [ $^{11}$ C]PK11195 in a rodent model of brain injury [8]. We have recently undertaken the first human study of [ $^{11}$ C]DPA-713, which

demonstrated a higher binding potential than [ $^{11}$ C]PK11195 [5]. However,  $^{11}$ C-labeled radiopharmaceuticals are limited to centers in which there is a cyclotron on site, can be difficult to handle due to the 20 min physical half-life of the radionuclide, and the synthesis of such compounds can be prohibitively costly for preclinical (small animal) imaging studies. Here we report the synthesis of the corresponding radioiodinated compound, [ $^{125}$ I]iodoDPA-713 [ $^{125}$ I]**1** for use in autoradiography and small animal imaging studies.

### Materials and methods

#### General

Chemicals and solvents obtained from commercial sources were analytical grade or better and used without further purification. Iodine-125 ( $^{125}$ I) was obtained as a 0.1 N solution of NaOH (high concentration) from MP Biomedicals (Solon, Ohio). Analytical thin-layer chromatography (TLC) was performed using Aldrich aluminum-backed 0.2 mm silica gel plates and visualized by UV light (254 nm) and  $I_2$ . Flash column chromatography was performed on silica gel (60 Å) from MP Biomedicals. Radio-HPLC purification was performed using a Waters (Milford, MA) system consisting of two Waters 510 pumps, a Waters 490E variable wavelength UV/Vis detector set at 254 nm, a BioScan FlowCount radioactivity detector, a Waters radial-PAK C18 reverse phase analytical column (8 × 100 mm) with  $H_2O/CH_3CN/TFA$  solvent systems, and WinFlow (LabLogic) chromatography software.  $^1H$  NMR was recorded on a Bruker (Billerica, MA) Ultrashield™ 400 MHz spectrometer. ESI mass spectra were obtained with a Bruker Daltonics Esquire

**Abbreviations:** TSPO, translocator protein; [ $^{125}$ I]iodoDPA-713, *N,N*-diethyl-2-[2-(3-[ $^{125}$ I]iodo-4-methoxyphenyl)-5,7-dimethylpyrazolo[1,5-*a*]pyrimidin-3-yl]acetamide; PK11195, *N*-butan-2-yl-1-(2-chlorophenyl)-*N*-methylisoquinoline-3-carboxamide; SPECT, single photon emission computed tomography

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300 plus spectrometer. Radioactivity was measured in a Capintec CRC-12 dose calibrator. The specific radioactivity was calculated as the radioactivity eluting at the retention time of [ $^{125}$ I]**1** during HPLC purification divided by the mass corresponding to the area under the curve of UV absorption.

### Chemical synthesis

*N,N*-Diethyl-2-[2-(4-hydroxy-phenyl)-5,7-dimethyl-pyrazolo[1,5-*a*]pyrimidin-3-yl]-acetamide **3**.  $\text{BBr}_3$  in  $\text{CH}_2\text{Cl}_2$  (3 mL, 3 mmol) was added dropwise to a solution of **2** (0.22 g, 0.58 mmol) in  $\text{CH}_2\text{Cl}_2$  at  $-78^\circ\text{C}$ . After stirring overnight the pH of the reaction mixture was adjusted to pH 8–9 using  $\text{NaHCO}_3$  and extracted with  $\text{CH}_2\text{Cl}_2$ . The organic layer was collected and dried over anhydrous  $\text{Na}_2\text{SO}_4$ . The solvent was removed under vacuum and the residue was purified by silica gel column chromatography ( $\text{CHCl}_3/\text{MeOH}$ , 40:1 (v/v), as eluent) to yield **3** (0.18 g, 85%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  1.06–1.18 (m, 6H), 2.54 (s, 3H), 2.73 (s, 3H), 3.34–3.51 (m, 4H), 3.96 (s, 2H), 6.49 (s, 1H), 6.79–6.82 (d,  $J = 8.7$  Hz, 2H), 7.61–7.64 (d,  $J = 8.4$  Hz, 2H). ESI MS  $m/z$ :  $[\text{M}+\text{H}]^+$ .  $\text{C}_{20}\text{H}_{25}\text{N}_4\text{O}_2$ : calculated 353.2, found 353.2.

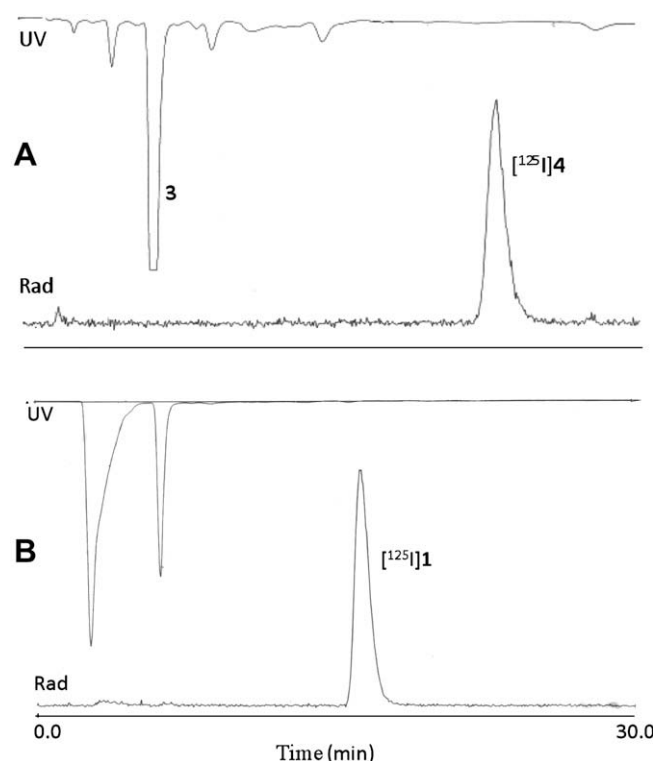
*N,N*-Diethyl-2-[2-(4-hydroxy-3-iodophenyl)-5,7-dimethylpyrazolo[1,5-*a*]pyrimidin-3-yl]acetamide **4**. To a solution of **3** (100 mg, 0.28 mmol) in MeOH (15 mL) was added NaI (52 mg, 0.35 mmol) and chloroamine-T hydrate (80 mg, 0.35 mmol). The reaction mixture was stirred for 1 h and then concentrated under vacuum. The residue was purified by column chromatography ( $\text{CH}_2\text{Cl}_2/\text{MeOH}$ , 20:1 (v/v), as eluent) to yield **4** (60 mg, 45% yield).  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 400 MHz)  $\delta$  1.16 (t,  $J = 7.2$  Hz, 3H), 1.29 (t,  $J = 7.2$  Hz, 3H), 2.57 (s, 3H), 2.76 (s, 3H), 3.44 (q,  $J = 7.2$  Hz, 2H), 3.59 (q,  $J = 7.2$  Hz, 2H), 3.97 (s, 2H), 6.80 (s, 1H), 6.92 (d,  $J = 8$  Hz, 1H), 7.57 (d,  $J = 8$  Hz, 1H), 8.01 (s, 1H). ESI MS  $m/z$ :  $[\text{M}+\text{H}]^+$ .  $\text{C}_{20}\text{H}_{24}\text{IN}_4\text{O}_2$ : calculated 479.1, found 479.1.

*N,N*-Diethyl-2-[2-(3-iodo-4-methoxyphenyl)-5,7-dimethylpyrazolo[1,5-*a*]pyrimidin-3-yl]acetamide (iodoDPA-713) **1**. To a solution of **4** (30 mg, 0.063 mmol) in DMF (5 mL) was added  $\text{K}_2\text{CO}_3$  (300 mg, 2.2 mmol) and MeI (100  $\mu\text{L} \times 2$  M, 0.2 mmol). The reaction was stirred overnight and the solvent was removed under vacuum and the residue was purified by silica gel column chromatography (EtOAc as eluent) to give **1** (29 mg, 0.60 mmol, 95%).  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 400 MHz)  $\delta$  1.15 (t,  $J = 7.2$  Hz, 3H), 1.29 (t,  $J = 7.2$  Hz, 3H), 2.57 (s, 3H), 2.77 (s, 3H), 3.43 (q,  $J = 7.2$  Hz, 2H), 3.58 (q,  $J = 7.2$  Hz, 2H), 3.92 (s, 3H), 3.97 (s, 2H), 6.80 (s, 1H), 7.06 (d,  $J = 8$  Hz, 1H), 7.74 (d,  $J = 8$  Hz, 1H), 8.09 (s, 1H). ESI MS  $m/z$ :  $[\text{M}+\text{H}]^+$ .  $\text{C}_{21}\text{H}_{26}\text{IN}_4\text{O}_2$ : calculated 493.1, found 493.1.

### Radiochemical synthesis

*N,N*-Diethyl-2-[2-(4-hydroxy-3-[ $^{125}$ I]-iodophenyl)-5,7-dimethylpyrazolo[1,5-*a*]pyrimidin-3-yl]acetamide [ $^{125}$ I]**4**. To a solution of 1 mg of **3** in 0.1 mL of  $\text{CH}_3\text{CN}$ , 0.1 mL of methanol and 0.1 mL of phosphate-buffered saline was added 0.2 mg of iodogen (Pierce, Rockford, IL), followed by 2 mCi of [ $^{125}$ I]NaI. The reaction mixture was incubated at room temperature for 1.5 h and purified by reverse phase HPLC using 70%  $\text{H}_2\text{O}/30\%$   $\text{CH}_3\text{CN}/0.1\%$  TFA with a flow rate of 2 mL/min on a Waters Radial-Pak analytical column (8  $\times$  10 mm). The retention time of [ $^{125}$ I]**4** was 22.8 min (Fig. 1A). The radioactive fraction corresponding to [ $^{125}$ I]**4** was collected, diluted with water and passed through a pre-conditioned C18 light Sep-Pak cartridge (Waters Corp, Milford, MA) eluted with 0.5 mL of ether. The ether was evaporated under a stream of nitrogen and the residue was used in the next step. Yield: 44% ( $n = 3$ ), 0.041 GBq (1.1 mCi).

*N,N*-Diethyl-2-[2-(3-[ $^{125}$ I]-iodo-4-methoxyphenyl)-5,7-dimethylpyrazolo[1,5-*a*]pyrimidin-3-yl]acetamide ([ $^{125}$ I]iodoDPA-713) [ $^{125}$ I]**1**. Compound [ $^{125}$ I]**4** 0.041 GBq (1.1 mCi) was dissolved in 0.2 mL of anhydrous DMF in a 2-mL V-vial. To this was added 10 mg of



**Fig. 1.** High-performance liquid chromatography (HPLC) traces in the synthesis of [ $^{125}$ I]**4** from **3** (A) and [ $^{125}$ I]iodoDPA-713 ([ $^{125}$ I]**1**) (B). Retention times for [ $^{125}$ I]**4** and [ $^{125}$ I]**1** were 22.8 and 15.5 min, respectively. The radiochemical yield of [ $^{125}$ I]**1** was  $44 \pm 6\%$  with a specific radioactivity of 51.8 GBq/ $\mu\text{mol}$  (1400 mCi/ $\mu\text{mol}$ ) and >99% radiochemical purity. Other yields and conditions are provided in Materials and methods.

$\text{K}_2\text{CO}_3$  followed by 10  $\mu\text{L}$  of 2 M MeI. The reaction mixture was stirred overnight and diluted with 0.2 mL of water and purified by reverse phase HPLC using 50%  $\text{H}_2\text{O}/50\%$   $\text{CH}_3\text{CN}/0.1\%$  TFA with a flow rate of 1 mL/min on a Waters Radial-Pak analytical column (8  $\times$  100 mm). The retention time of [ $^{125}$ I]**1** was 15.5 min (Fig. 1B). The radioactive fraction corresponding to [ $^{125}$ I]**1** was collected, diluted with water and passed through a pre-conditioned C18 light Sep-Pak and eluted with 0.5 mL of ethanol. Yield: 0.035 GBq (0.95 mCi), 86% from [ $^{125}$ I]**4** and 47% from **3**. Specific radioactivity was 51.8 GBq/ $\mu\text{mol}$  (1400 Ci/mmol).

### In vitro autoradiography

Fresh-frozen brains were sectioned (20  $\mu\text{m}$ ) on a freezing cryostat in the horizontal plane. Brain sections were thaw-mounted onto poly-L-lysine-coated slides (Sigma) and stored at  $-20^\circ\text{C}$  until used. Autoradiography using [ $^{125}$ I]**1** was performed on adjacent brain sections using the following procedures. Slides were thawed and dried at  $37^\circ\text{C}$  for 30 min and prewashed in 50 mM Tris–HCl buffer (pH 7.4) for 5 min at room temperature. Sections were then incubated in 1.4 nM [ $^{125}$ I]**1** in 50 mM Tris–HCl buffer for 30 min at room temperature. For non-specific binding, adjacent sections were incubated in the presence of 10  $\mu\text{M}$  racemic PK11195. The reaction was terminated by two 3 min washes in cold buffer ( $4^\circ\text{C}$ ) and two dips in cold deionized water ( $4^\circ\text{C}$ ). Sections were air-dried and apposed to Kodak Bio-Max MR film for 1 h. Images were acquired using the MCID image analysis software (InterFocus Imaging Ltd., Cambridge, England).

### SPECT-CT imaging

Eight- to 10-week-old female CD-1 mice (Charles River Labs) were imaged. Mice were anesthetized by brief isoflurane sedation.

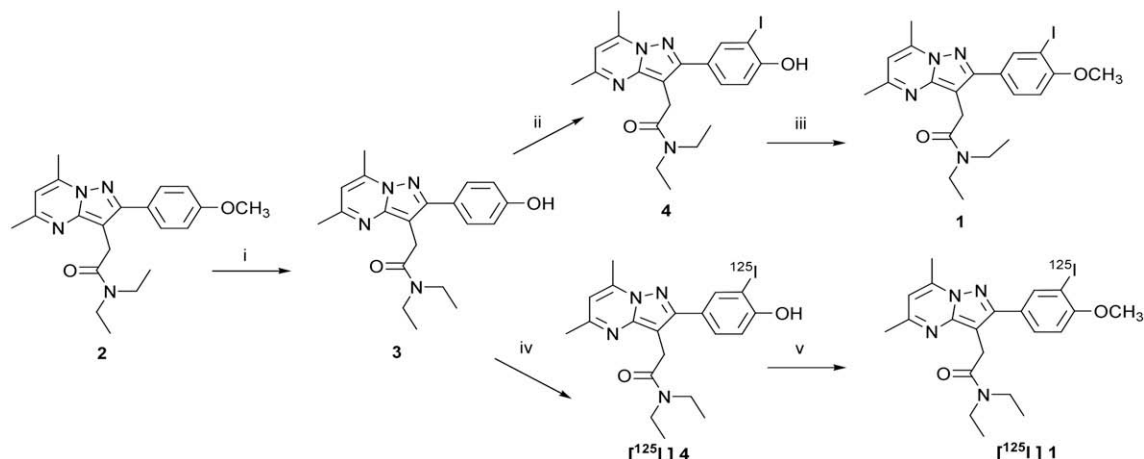
While anesthetized, intranasal instillation was conducted by placing 10  $\mu\text{g}/60\ \mu\text{L}$  (167  $\mu\text{g}/\text{mL}$ ) of lipopolysaccharide (LPS) onto the nares [9]. The 60  $\mu\text{L}$  sample was applied onto the nares as three 20  $\mu\text{L}$  drops. Phosphate-buffered saline (PBS) was administered in a similar fashion and used as the control.

For single photon emission computed tomography-computed tomography (SPECT-CT) imaging 18.5 MBq (500  $\mu\text{Ci}$ ) of [ $^{125}\text{I}$ ] **1** was injected *via* the tail vein into the LPS and PBS treated mice and imaged 1 h later, which corresponded to 24 h after the administration of LPS or PBS. That time point was chosen since it represents the time at which there is maximum inflammation in the lungs, as determined by a time course study with histologic correlation (data not shown). Each mouse was anesthetized with isoflurane and maintained under 1–2% isoflurane in oxygen. The mouse was positioned on the X-SPECT (Gamma Medica, Northridge, CA) gantry and was scanned using two opposing low energy pinhole collimators (Gamma Medica) rotating through 360° in 3° increments for 20 s per increment. Images were reconstructed using LumaGem software that accompanies the X-SPECT. Immediately following SPECT acquisition, the mice were then scanned by CT over a 3-cm field-of-view using a 600- $\mu\text{A}$ , 50 kV beam. The SPECT and CT data were then co-registered using the X-SPECT software and displayed using AMIDE (<http://amide.sourceforge.net/>). Data was reconstructed using the ordered subsets-expectation maxi-

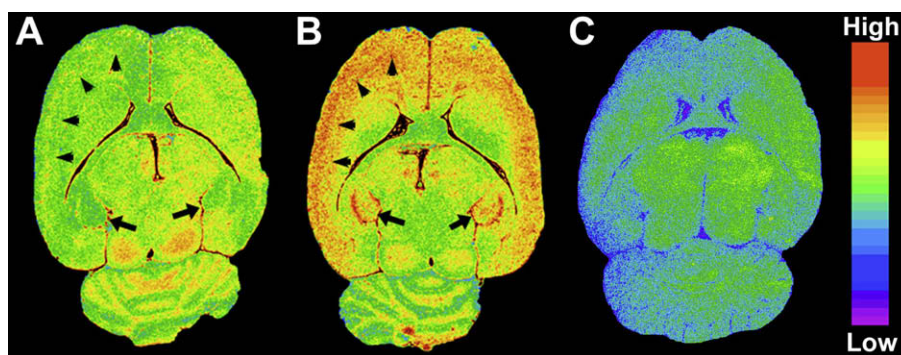
mization (OS-EM) algorithm. The signal to background ratio was calculated from the images by drawing regions of interest over the lungs (signal) and muscle, (background).

## Results and discussion

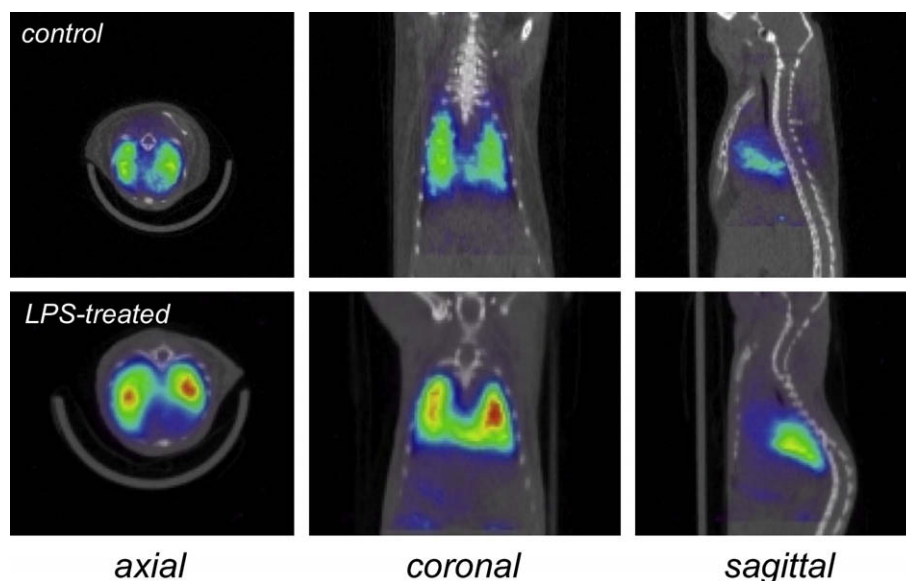
*N,N*-Diethyl-2-[2-(3-iodo-4-methoxyphenyl)-5,7-dimethylpyrazolo[1,5-*a*]pyrimidin-3-yl]acetamide (iodoDPA-713) **1** was synthesized according to Scheme 1 in three steps from *N*-diethyl-2-[2-(4-methoxy-phenyl)-5,7-dimethylpyrazolo[1,5-*a*]pyrimidin-3-yl]-acetamide **2** (DPA-713). Compound **2** was prepared by the procedure of James et al. [7]. Attempts were made to iodinate **2** directly to **1** using NaI and *N*-chlorosuccinimide or chloramine-T as oxidants in acidic solvents (concentrated trifluoroacetic acid or trifluoromethanesulfonic acid). However, those conditions produced no or extremely low yields of **1**. Consequently, **2** was demethylated by reaction with  $\text{BBr}_3$  in methylene chloride to form the more reactive phenol derivative *N,N*-diethyl-2-[2-(4-hydroxy-phenyl)-5,7-dimethyl-pyrazolo[1,5-*a*]pyrimidin-3-yl]-acetamide **3**. Compound **3** was smoothly iodinated using NaI with chloramine-T to form *N,N*-diethyl-2-[2-(4-hydroxy-3-iodophenyl)-5,7-dimethylpyrazolo[1,5-*a*]pyrimidin-3-yl]acetamide **4**. Finally, *O*-methylation of **4** gave **1**, which could then serve as the cold standard for high-performance liquid chromatography (HPLC) for radiosynthesis.



**Scheme 1.** Synthesis of *N,N*-diethyl-2-[2-(3-iodo-4-methoxyphenyl)-5,7-dimethylpyrazolo[1,5-*a*]pyrimidin-3-yl]acetamide (iodoDPA-713) **1** and *N,N*-diethyl-2-[2-(3-[ $^{125}\text{I}$ ]-iodo-4-methoxyphenyl)-5,7-dimethylpyrazolo[1,5-*a*]pyrimidin-3-yl]acetamide ([ $^{125}\text{I}$ ]iodoDPA-713 [ $^{125}\text{I}$ ] **1**); (i) boron tribromide, dichloromethane,  $-78\ ^\circ\text{C}$ , overnight, 85%, (ii) chloramine-T, phosphorus buffered saline (pH 7.4), methanol, 1 h, 45%, (iii) methyl iodide, potassium carbonate, dimethyl formamide, overnight, 95%, (iv) iodogen, [ $^{125}\text{I}$ ]NaI,  $\text{CH}_3\text{CN}/\text{MeOH}/\text{PBS}$  1.5 h, 47% ( $n = 3$ ), (v)  $\text{K}_2\text{CO}_3/\text{MeI}/\text{DMF}$ , 92% ( $n = 3$ ) 51.8 GBq/ $\mu\text{mol}$  (1400 Ci/mmol).



**Fig. 2.** Autoradiographic images of [ $^{125}\text{I}$ ]iodoDPA-713 [ $^{125}\text{I}$ ] **1** to TSPO in the rat brain. Representative horizontal images of [ $^{125}\text{I}$ ] **1** binding to TSPO in a normal rat brain (A) and in a neurotoxicant-injected rat brain (B) relative to the control brain (A). There is a significant increase in TSPO levels in the cerebral cortex (arrowheads) and hippocampus (arrows) in the neurotoxicant-injected rat brain (B) relative to the control brain (A). The image in (C) is representative of [ $^{125}\text{I}$ ] **1** non-specific binding using 10  $\mu\text{M}$  R-PK11195 as the blocking agent in a neurotoxicant-treated rat.



**Fig. 3.** SPECT-CT imaging at 1 h postinjection. The lung signal to background ratio in the control mouse (top row) was 14.6 to 1 and that in the LPS treated mouse (bottom row) was 21.2 to 1.

The synthesis of [ $^{125}\text{I}$ ]**1** proceeded with an average radiochemical yield of  $44 \pm 6\%$  by reaction of **3** with iodogen (Scheme 1) followed by methylation of the radioiodinated product. Compound [ $^{125}\text{I}$ ]**1** was produced in specific radioactivities of  $51.8 \text{ GBq}/\mu\text{mol}$  ( $1400 \text{ mCi}/\mu\text{mol}$ ) in  $>99\%$  radiochemical purity.

Compound [ $^{125}\text{I}$ ]**1** underwent two preliminary tests to determine its utility for imaging inflammation. The first study involved a model of brain inflammation in which rats were exposed to a seizure-inducing neurotoxicant (Fig. 2). Note that higher levels of [ $^{125}\text{I}$ ]**1** were present in the neurotoxicant-treated rat brain (Fig. 2B) than in control brain (Fig. 2A). Exposure of the tissue specimens to [ $^{125}\text{I}$ ]**1** to generate these images required only 60 min, as opposed to 4–5 weeks often needed for tritium-labeled compounds when exposing the samples to film. Rapid images can also be obtained with tritium-labeled compounds, including [ $^3\text{H}$ ]DPA-713, using an automated system such as a Beta-Imager [10], however the resolution of such images is generally inferior to that which can be obtained by exposing the tissue slices to film. Also note that uptake of [ $^{125}\text{I}$ ]**1** could be almost completely blocked in the neurotoxicant-treated rat upon treatment with PK11195 (Fig. 2C), demonstrating that most binding seen in this *in vitro* study was specific for TSPO. An *in vivo* small animal SPECT-CT study demonstrated 1.5-fold higher uptake of [ $^{125}\text{I}$ ]**1** in inflamed mouse lungs than in normal lungs (Fig. 3). Although only one result is presented here, that was a consistent finding. Together these two preliminary studies suggest that [ $^{125}\text{I}$ ]**1** may be useful to study pre-clinical models of inflammation. Compound [ $^{125}\text{I}$ ]**1** is particularly relevant as it is patterned after compounds that are currently undergoing early clinical testing [5,6].

## Conclusions

[ $^{125}\text{I}$ ]iodoDPA-713 ([ $^{125}\text{I}$ ]**1**) can be readily synthesized from DPA-713 in high radiochemical yield and specific radioactivity. It demonstrates specific binding to TSPO *in vitro* and *in vivo*, showing a higher level of uptake in inflamed than in the corresponding normal tissue. This agent provides a convenient and inexpensive alter-

native to other radiolabeled analogs of the pyrazolopyrimidine series for autoradiographic and preclinical imaging studies.

## Acknowledgments

We thank Jennifer McGlothlan for expertise in autoradiography and James Fox and Gilbert Green for providing the *in vivo* images. We also thank the Dana Foundation and NIH Grants CA92871 and ES07062 for financial support. The funding sources had no impact on the scientific content of this contribution.

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