

## Supporting Information

### Synthesis and evaluation of methylated arylazepine compounds for PET imaging of 5-HT<sub>2c</sub> receptors

Michael L. Granda, Stephen M. Carlin, Christian K. Moseley, Ramesh Neelamegam, Joseph B. Mandeville, and Jacob M. Hooker\*

*Athinoula A. Martinos Center for Biomedical Imaging, Department of Radiology, Massachusetts General Hospital, Harvard Medical School, Charlestown, Massachusetts 02129, United States*

#### ***General methods and materials***

All reagents and solvents were of ACS-grade purity or higher and used without further purification. Reactions were performed manually, not including the automated synthesis of [<sup>11</sup>C]**5**, using standard technique including inert atmosphere of nitrogen with standard Schlenk technique where necessary. NMR data were recorded on a Varian 500 MHz 11.7 T magnet and reported in ppm units downfield from trimethylsilane. Analytical separation was conducted on an Agilent 1100 series HPLC fitted with a diode-array detector, quaternary pump, vacuum degasser, and autosampler. Mass spectrometry data were recorded on an Agilent 6310 ion trap mass spectrometer (ESI source) connected to an Agilent 1200 series HPLC with quaternary pump, vacuum degasser, diode-array detector, and autosampler. Analytical separation by HPLC was achieved by a gradient of acetonitrile in 0.1 M ammonium formate (2% for 0-3 min, 2%-30% for 3-13 min, 30%-90% for 13-16 min in terms of acetonitrile concentration % v/v). Purification by flash chromatography was conducted on an ISCO CombiFlash Companion unit.

***tert-Butyl-10-chloro-8,9-dihydro-5H-pyrazolo[1',5':1,2]pyrimido[4,5-d]azepine-7(6H)-carboxylate (1b)***

**1** (1.66 g, 7.5 mmol) was dissolved in dichloromethane (10 mL) and combined with triethylamine (2.61 mL, 18.7 mmol) and di-tert-butyl dicarbonate (1.79 g, 8.2 mmol). The mixture was stirred at room temperature for 2 h and quenched with water (10 mL). The mixture was extracted with dichloromethane (DCM, 2 x 20 mL), and the organic layer was dried over MgSO<sub>4</sub>. The product was isolated using a flash chromatography gradient in hexanes:ethyl acetate (2:0 to 1:1). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 8.11 (d, *J* = 2 Hz, 1H), δ 6.64 (d, *J* = 2 Hz, 1H), δ 3.16 (m, 4H), δ 3.15 (m, 4H), δ 1.49 (s, 9H). LCMS (M+1) found 323.2. Expected = 323.12.

***10-(Azetidin-1-yl)-6,7,8,9-tetrahydro-5H-pyrazolo[1',5':1,2]pyrimido[4,5-d]azepine (2)***

**1b** (0.4 g, 1.24 mmol) was dissolved in anhydrous ethanol (20 mL). Azetidine hydrochloride (0.1261 g, 1.36 mmol) was added, and the mixture was heated under reflux at 80°C. DIPEA (1.1 mL, 6.4 mmol) was added over 2 h, and the reaction allowed to stir for 24 h. The reaction was then quenched with water (20 mL) and extracted with ethyl acetate (2 x 20 mL). The organic layer was dried under MgSO<sub>4</sub> and concentrated under reduced-pressure rotary evaporation. The crude product was separated using a flash chromatography gradient in hexanes:ethyl acetate (2:0 to 1:1). To Boc-deprotect the azepine, the product was dissolved in DCM (4 mL) with trifluoroacetic acid (1 mL) for 2 h at room temperature. <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD): δ 7.81 (d, *J* = 2 Hz, 1H), δ 6.2 (d, *J* = 2 Hz, 1H), δ 3.30 (m, 4H), δ 3.18 (m, 6H), δ 3.07 (m, 2H), δ 2.38 (quin, *J* = 23.5 Hz, 2H), δ 2.10 (s, 1H). LCMS (M+1) found 244.2. Expected = 244.15.

***10-Methoxy-7-methyl-6,7,8,9-tetrahydro-5H-pyrazolo[1',5':1,2]pyrimido[4,5-d]azepine (3)***

Methanol (8 uL, 0.2 mmol) was combined with sodium hydroxide (7.88 mg, 0.2 mmol) in acetonitrile (1.5 mL) under reflux at 80°C for 1 h. Compound **1b** (30 mg, 0.093 mmol) was added and

stirred for 4 h. The reaction was quenched with H<sub>2</sub>O (3 mL) and extracted with ethyl acetate (3 x 5 mL). The organic layer was dried over MgSO<sub>4</sub> and concentrated *in vacuo*. The product was purified using a flash chromatography gradient in hexanes:ethyl acetate (100:0 to 5:95). Deprotection was as described for compound **2**. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 8.05 (d, *J* = 2 Hz, 1H), δ 6.55 (d, *J* = 2 Hz, 1H), δ 4.33 (s, 3H), δ 3.63 (m, 4H), δ 2.33 (m, 4H), δ 2.0 (s, 1H). LCMS (M+1) found 219.2. Expected = 219.12

***10-Phenoxy-6,7,8,9-tetrahydro-5H-pyrazolo[1',5':1,2]pyrimido[4,5-d]azepine (4)***

See procedure for **3**. Phenol (18.8 mg, 0.2 mmol) was used instead of methanol. <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD): δ 8.043 (s, 1H), δ 7.356 (t, *J* = 15Hz, 2H), δ 7.161 (t, *J* = 14Hz, 1H), δ 7.69 (m, 2H), δ 6.67 (d, *J* = 2Hz, 1H), δ 3.48 (m, 4H), δ 3.30 (m, 4H), δ 1.57 (s, 1H). MS (M+1) found 281.2, calculated 281.14.

***10-(Azetidin-1-yl)-7-methyl-6,7,8,9-tetrahydro-5H-pyrazolo[1',5':1,2]pyrimido[4,5-d]azepine (5)***

**2** (60 mg, 0.25 mmol) was dissolved in methanol (2 mL), and combined with formaldehyde (1 mL, 35% in methanol). Sodium cyanoborohydride (78 mg, 1.25 mmol) was added, followed by glacial acetic acid (0.5 mL). The mixture was stirred for 2 h at 35°C, and monitored by HPLC. Sodium borohydride (15 mg, 0.4 mmol) was added and the mixture slowly quenched with a NaOH solution (1 M, 3 mL). The product was extracted with diethyl ether (3 x 10 mL) with addition of 0.5 g sodium chloride, and dried over MgSO<sub>4</sub>. The crude mixture was purified by flash chromatography in CH<sub>2</sub>Cl:MeOH (10:0 to 4:6). LCMS (M+1) found 258.2. Expected = 258.17.

***7-Methyl-10-phenoxy-6,7,8,9-tetrahydro-5H-pyrazolo[1',5':1,2]pyrimido[4,5-d]azepine (7)***

See procedure for **5**. **4** (70 mg, 0.25 mmol) was used instead of **2**.

***10-Chloro-7-methyl-6,7,8,9-tetrahydro-5H-pyrazolo[1',5':1,2]pyrimido[4,5-d]azepine (8)***

See procedure for **5. 1** (56 mg, 0.25 mmol) was used instead of **2**.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.26 (d,  $J = 1\text{Hz}$ , 1H),  $\delta$  6.77 (d,  $J = 1\text{Hz}$ , 1H),  $\delta$  3.34 (m, 8H),  $\delta$  2.48 (s, 3H). LCMS (M+1) found 237.1. Calculated 237.09

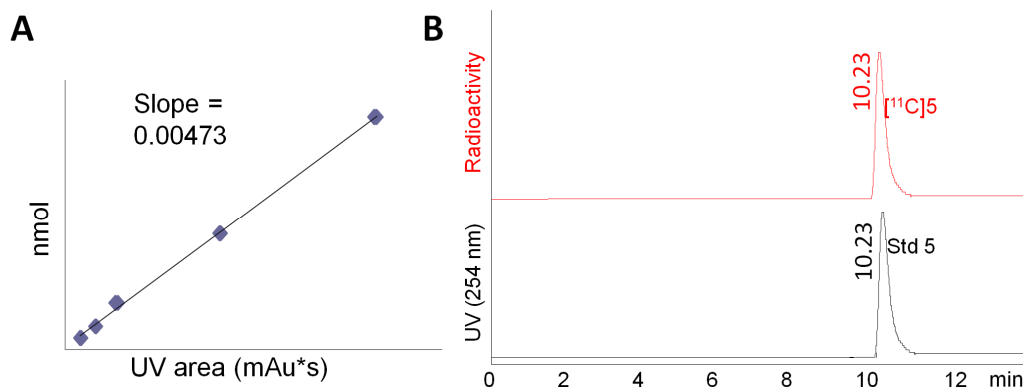
### ***Production of [ $^{11}\text{C}$ ]5***

Radioactivity was produced by an Eclipse HP 11 MeV cyclotron (Siemens, Munich, Germany) configured with a  $^{11}\text{C}$  gas target.  $^{11}\text{CO}_2$  was transferred to a TRACERlab FX-MeI unit (General Electric, Fairfield, CT), then reduced to  $^{11}\text{CH}_4$  and iodinated to produce high specific activity [ $^{11}\text{C}$ ]-iodomethane. The [ $^{11}\text{C}$ ]-iodomethane was transferred to a TRACERlab FX-M unit (General Electric, Fairfield, CT) preloaded with **2** (1.5 mg) dissolved in dimethyl sulfoxide (250  $\mu\text{L}$ ). The mixture was stirred at  $95^\circ\text{C}$  for 5 min, quenched with water (1.25 mL) and sparged of volatile activity with helium. The crude mixture was loaded onto a semi-preparative HPLC column (Phenomenex Gemini NX-C18 250x10mm, 5  $\mu\text{m}$ , 5mL/min) and separated using a mobile phase of ammonium formate (0.1M):acetonitrile (86:14). [ $^{11}\text{C}$ ]**5** was detected by monochromatic UV detector ( $\lambda=254\text{nm}$ ) and in-line NaI gamma detector. The product peak appeared 10.5 min after injection onto the HPLC, and diverted to a collection bulb pre-filled with water (20 mL). This mixture was delivered to a Sep-Pak SPE C18 (Waters Corp, Milford, MA) cartridge that was primed with ethanol (5 mL) and sterile water (40 mL). The cartridge was washed with sterile water (5 mL) to remove residual HPLC solvent and the product collected with ethanol (1 mL) followed by injectable saline (9 mL, USP grade).

### ***Specific Activity of [ $^{11}\text{C}$ ]5***

Specific radioactivity (SA) of [ $^{11}\text{C}$ ]**5** was calculated from an analytical HPLC sample of 100  $\mu\text{L}$ . A calibration curve of known mass quantity versus HPLC peak area (254 nm) was used to calculate the mass concentration of the 100  $\mu\text{L}$ . SA was determined using the mass concentration value determined for the formulated [ $^{11}\text{C}$ ]**5** solution, volume, and measured radioactivity. The identity of the mass peak

assigned to [ $^{11}\text{C}$ ]5 was supported by a replicated injection where [ $^{11}\text{C}$ ]5 was treated with non-radioactive 5. The calibration curve used and co-injection HPLC data appear in Figure S1.



**Figure S1. Analysis of [ $^{11}\text{C}$ ]5** (a) Mass/UV calibration curve. Five concentrations (0.5, 1.0, 2.0, 5.0 and 10.0  $\mu\text{M}$ ) of standard 5 were made and 100  $\mu\text{L}$  ( $n=3$ ) of each concentrations were injected into analytical HPLC column using UV (254 nm) (Agilent; Gemini C18 [250 $\times$ 4.6 mm]). Flow rate: 2 mL/min; Slope = 0.00473. (b) Aliquots of the formulated [ $^{11}\text{C}$ ]5 solution mixed with cold standard 5 were analyzed by analytical HPLC column using UV (254 nm) and gamma detectors in-line (Agilent; Gemini C18 [250 $\times$ 4.6 mm]); gamma trace offset by -0.2 min to compensate for dead volume between detectors; mobile phase: Analytical separation by HPLC was achieved by a gradient of acetonitrile in 0.1 M ammonium formate (2% for 0-3 min, 2%-30% for 3-13 min, 30%-90% for 13-16 min in terms of acetonitrile concentration % v/v); flow rate 2 mL/min; retention time: 9.6 min for UV (254 nm) and radioactivity spectra respectively.

### ***Rodent PET/CT acquisition***

Male Sprague-Dawley rats were utilized in pairs, anesthetized with inhalational isoflurane (Forane) at 3% in a carrier of 2 L/min medical oxygen and maintained at 2% isoflurane for the duration of the scan. The rats were arranged head-to-head in a Triumph Trimodality PET/CT/SPECT scanner (Gamma Medica, Northridge, CA) featuring a PET resolution of approximately 1 mm. Rats were injected 10 min prior to the start of PET acquisition with ketanserin, altanserin, 5, or vehicle via a lateral tail vein catheterization. Dynamic PET acquisition lasted for 80 min and was followed by computed tomography (CT) for anatomic coregistration. The rats were sacrificed by an intravenous administration of 50 mg/kg sodium pentobarbital (Nembutal) and immediately dissected. Radioactivity in individual organs as measured in a WIZARD<sup>2</sup> Automatic Gamma Counter (Perkin-Elmer, Waltham, MA) was normalized to the mass of the organ tissue sample.

### ***Baboon MR-PET acquisition***

A female *Papio Anubis* baboon, deprived of food for 12 h prior to the study, was administered intramuscular ketamine (10 mg/kg) and intubated. For maintenance of anesthesia throughout the study, the baboon was provided 1%-4% isoflurane (Forane) in a mixture of medical oxygen and nitrogen. The baboon was catheterized antecubitally for radiotracer injection and a radial arterial line was placed for metabolite analysis.

MR-PET images were acquired in a Biograph mMR scanner (Siemens, Munich, Germany), with a PET resolution of 5 mm and field of view of 59.4 cm and 25.8 cm (transaxial and axial, respectively). Dynamic PET image acquisition was initiated followed by administration of the radiotracer in a homogenous solution of 10% ethanol and 90% isotonic saline. Blood sampling occurred nominally every 10 sec for 3 min for measurement of arterial input of the radiotracer, followed by sampling at 5, 10, 20, 30, 45, 60, and 80 min time points for metabolite analysis. An MEMPRAGE sequence began after 30 min of the baseline scan for anatomic coregistration. To characterize the specific binding of [ $^{11}\text{C}$ ]5, a second imaging experiment was carried out in which ketanserin (1 mg/kg) was administered intravenously 10 min before the start of acquisition. Both scans were carried out in the same animal on the same day, separated by 2.5 h. In both scans,  $171.86 \pm 0.185$  MBq of [ $^{11}\text{C}$ ]5 was administered to the baboon.

### ***Plasma and metabolite analysis***

Arterial samples collected during imaging from the baboon were centrifuged to obtain plasma, which was then removed and placed in an automated gamma counter that was calibrated to the PET scanner. Metabolite analysis was conducted on a custom automated robot fitted with Phenomenex SPE Strata-X 500 mg solid phase extraction cartridges that were primed with ethanol (2 mL) and deionized water (20 mL). Protein precipitation was achieved by addition of plasma (300  $\mu\text{L}$ ) to acetonitrile (300

μL), which was centrifuged for 1 min to obtain protein-free plasma (PFP). 300 μL of PFP/acetonitrile solution was diluted into deionized water (3 mL), loaded onto the C18 cartridge, and removed of polar metabolites with 100% water. Next, a series of extractions were performed using water and acetonitrile in quantities: 2 x 95:5, 90:10, 85:15, 80:20, and 100% acetonitrile at a volume of 4 mL. A control experiment was performed before metabolite analysis to determine the retention of the parent compound by injection of a small amount of [<sup>11</sup>C]**5** onto a test series of extraction cartridges. Each sample was counted in a WIZARD<sup>2</sup> Automatic Gamma Counter to determine the presence of radiolabeled metabolites.

### ***Injection solution preparation***

Ketanserin tartrate and altanserin hydrochloride hydrate were obtained from Sigma-Aldrich (St. Louis, MO) and utilized without further purification. **5** was produced as specified and purified by flash chromatography to at least 95% as determined by HPLC. The solid was weighed into a dram vial, dissolved in a minimum of ethanol and diluted with sterile isotonic saline to a final ethanol concentration of 10% v/v. The solutions were titrated with 0.5 μL aliquots of acetic acid and vortexed until the solution became clear. Before injection, the solutions were passed through a sterile Millex-LG syringe-driven filter unit (Millipore, Billerica, MA).

### ***Image reconstruction and analysis***

Dynamic data from the PET scans were recorded in list mode and corrected for attenuation. Baboon data were reconstructed using a 3D-OSEM method resulting in a full width at half-maximum resolution of 4 mm. Rat PET data were reconstructed using a 3D-MLEM method resulting in a full width at half-maximum resolution of 1 mm. Reconstructed images were exported from the scanner in DICOM format along with an anatomic CT for rodent studies and MRI for baboon scans. These files were imported to PMOD (PMOD Technologies, Ltd.) and manually coregistered using six degrees of

freedom. Volumes of interest (VOIs) were drawn manually as spheres in brain regions guided by high resolution structural images (MRI for baboon and CT for rats) and summed PET data, with a radius no less than double that of the PET voxel size to minimize partial volume effects (4 mm for baboon and 1 mm for rodent scans). A common VOI mask was applied to both baboon scans. Time-activity curves (TACs) were exported in terms of decay corrected activity per unit volume at specified time points with gradually increasing intervals. The TACs were expressed as percent injected dose per unit volume for analysis.