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^{11}C-labeling and preliminary evaluation of pimavanserin as a 5-HT$_{2A}$ receptor PET-radioligand

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ABSTRACT

Pimavanserin is a selective serotonin 2A receptor (5-HT$_{2A}$R) inverse agonist that has shown promise for treatment of psychotic symptoms in patients with Parkinson's disease. Here, we detail the ^{11}C-labeling and subsequently evaluate pimavanserin as a PET-radioligand in pigs. [^{11}C]Pimavanserin was obtained by N-methylation of an appropriate precursor using [^{11}C]MeOTf in acetone at 60 °C giving radiochemical yields in the range of 1–1.7 GBq (n = 4). In Danish Landrace pigs the radio ligand readily entered the brain and displayed binding in the cortex in accordance with the distribution of 5-HT$_{2A}$Rs. However, this binding could not be blocked by either ketanserin or pimavanserin itself, indicating high nonspecific binding. The lack of displacement by the 5-HT$_2$R antagonist and binding in the thalamus suggests that [^{11}C]pimavanserin is not selective for the 5-HT$_{2A}$R in pigs.

The serotonin 2A receptor (5-HT$_{2A}$R) is the most abundant excitatory 5-HT receptor in the human brain. Stimulation of 5-HT$_{2A}$R with agonists such as LSD and mescaline leads to hallucinogenic effects, whereas 5-HT$_{2A}$R antagonism is a hallmark of atypical antipsychotics.[1] Positron emission tomography (PET) can be used to image specific proteins and processes in the human brain, and is a widely used tool to quantify differences in receptor binding, for example, between patient and control groups and to quantify receptor occupancy of drugs. Thus, a PET-radioligand that can be used to visualize the 5-HT$_{2A}$R system would be a very valuable tool to elucidate the role of this receptors psychophysiological functions and involvement in diseases.

Several compounds have been evaluated as PET-radioligands for the 5-HT$_{2A}$R most of them being antagonists and more recently also full or partial agonists.[2–10] In theory, agonist tracers should selectively label the activated states of the receptors, whereas antagonists bind to all receptor states. Inverse agonists have the potential to selectively label the inactivated states of receptors, and thus provide an additional imaging tool.

Pimavanserin (**1**) is a selective 5-HT$_{2A}$R inverse agonist that has proven effective in the treatment of psychotic symptoms emerging in Parkinson's disease (PDP); which affects up to 50% of these patients during their lifetime.[11,12] Clinical trials have shown effect of **1** on PDP as well as a good tolerability with a safety profile similar to placebo.[11,14,15]

Pimavanserin has very high affinity for the 5-HT$_{2A}$R (pK_i = 9.3) in membrane binding assays and even higher affinity in whole cell studies (pK_i = 9.70) and the pK_i for the 5-HT$_{2C}$R in the same assays are 8.80 and 8.00, respectively.[13] Since **1** shows no measurable affinity towards other G-protein coupled receptors it has a promising profile for selective imaging of the 5-HT$_{2A}$R making it an interesting candidate for a PET radioligand. Furthermore, a PET-radioligand based on **1** could be useful not only to study the 5-HT$_{2A}$R, but also give insights to the pharmacokinetics of this novel drug.

The structure of **1** (see Fig. 1) offers two sites amenable to either ^{11}C- or ^{18}F-labeling. [^{11}C]**1** could be accessible via labeling of the corresponding secondary amine, whereas [^{18}F]**1** (in analogy to

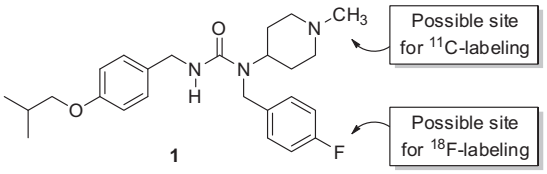

Figure 1. Structure of pimavanserin (**1**) showing amenable sites for radiolabeling.

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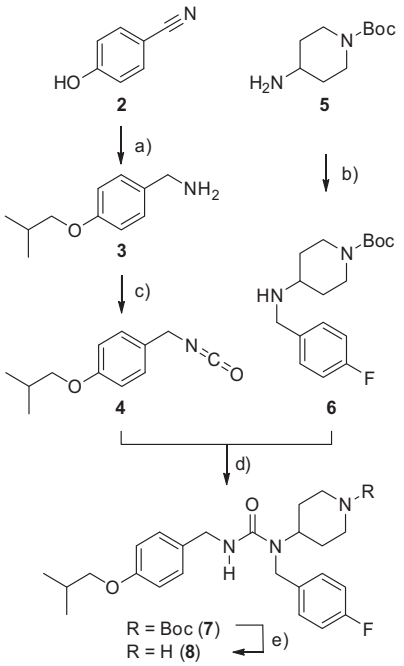


Scheme 1. Precursor synthesis. Reagents and conditions: (a) (i) 1-bromo-2-methylpropane, K_2CO_3, acetone, 60 °C, 48 h, 76%, (ii) $LiAlH_4$, THF, 0 °C, 54%. (b) 4-Fluorobenzaldehyde, $NaCNBH_3$, MeOH, 18 h. (c) Triphosgene, Et_3N, CH_2Cl_2, rt, 30 min. (d) Et_3N, CH_2Cl_2, 1 h. 81% (from **3**). (e) TFA, CH_2Cl_2, 98%.

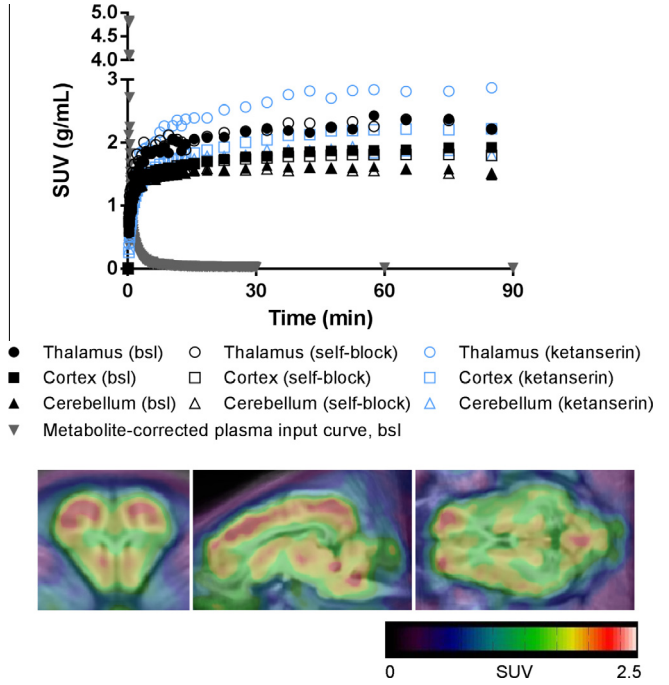


Figure 2. Top: time-activity curves for the indicated brain regions. Filled symbols represent the baseline (bsl) scan, open black symbols represent the self-block scan, and open blue symbols represent the ketanserin pretreatment scan. SUV: standardized uptake value. Bottom: coronal, sagittal and transverse (left to right) summed PET images (baseline scan, 0–90 min, 3 mm Gaussian filtering) of [^{11}C]**1** in the pig brain.

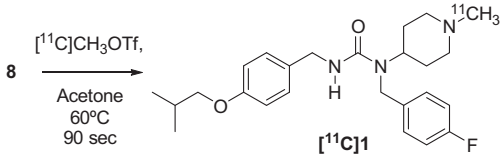
the transformation detailed in Scheme 1) could be available from [^{18}F]4-fluorobenzaldehyde. We chose to focus on accessing the ^{11}C-derivative first since this approach obviates the development of a multi-step radiochemical route.

The required precursor was synthesized as outlined in Scheme 1. Alkylation followed by reduction of 4-hydroxybenzonitrile (**2**) gave the known primary amine **3**. Subsequent treatment with triphosgene provided the corresponding isocyanate **4**, which was immediately reacted with **6**[16]—available in one step from **5** via reductive amination with 4-fluorobenzaldehyde. This sequence provided **7** in 87% yield. Removal of the Boc-group in **7** gave the desired precursor **8** in 98% yield (Scheme 2).[17]

[^{11}C]**1** was obtained from **8** and [^{11}C]MeOTf in acetone at 60 °C for 90 s. Preparative HPLC-purification provided 1–1.7 GBq [^{11}C]**1** in >98% radiochemical purity and with a specific activity of 34–80 GBq/μmol (n = 4), see Scheme 1.[18]

[^{11}C]**1** was injected intravenously into a Danish Landrace pig.[19] During the acquisition time, high brain uptake was observed but with relatively slow tracer kinetics resulting in very limited net wash-out of the radio ligand.

Due to constant availability of radioligand in the blood (see Fig. 2), it is not surprising that the radioligand displays near irreversible binding in the brain. The slow kinetics is, however, likely to become a problem for compartmental kinetic modeling. With the steady state concentrations of radioactivity the distribution volume (V_T) can be calculated as the ratio between plasma and the brain region in question. In principle these steady state levels



Scheme 2. ^{11}C-labeling of pimavanserin (**1**).

of [^{11}C]**1** in the plasma and brain open for the possibility of only scanning between 60 and 90 min after the bolus injection of radioligand.

Moderate binding was found in the cortex (V_T = 107 mL/cm^3) where the density of 5-HT_{2A}R is the highest[20] and lower binding was observed in the cerebellum (V_T = 89 mL/cm^3). Using the 5-HT_{2A}R specific radioligand [^{3}H]MDL100907 it was found that the density of 5-HT_{2A}R in the pig cerebellum was 50% lower than the density of receptors in the frontal cortex. Thus it was not unexpected to observe binding in the cerebellum.[21] Unexpectedly, the highest binding was seen in the thalamus (V_T = 130 mL/cm^3), which may be due to binding to one or more off targets. To test for specific binding of [^{11}C]**1**, a second imaging study was conducted with the co-administration of [^{11}C]**1** and unlabeled **1** (0.26 mg/kg, iv). We expected that the unlabeled **1** would saturate the receptors in the brain to decrease the binding of [^{11}C]**1** to its target. However, no changes in the radioligand distribution or pharmacokinetics were observed in any brain region (Fig. 2), suggesting that [^{11}C]**1** exhibits high non-specific binding. A pretreatment experiment with the 5-HT_2R antagonist ketanserin did not result in any noticeable blocking effect either. Brain uptake increased slightly, which was puzzling since the metabolite corrected plasma input curves were essentially identical.

[^{11}C]**1** was subject to rapid metabolism resulting in two metabolites, both of which were more polar than the parent compound, see Figure 3.[22] After 10 min, less than 70% of the parent compound was present. No difference in rate of metabolism was seen between the baseline and self-block experiment. The rate of metabolism was however faster when the animal was pretreated with ketanserin, likely due to peripheral blocking of receptors resulting in the radioligand being more readily available for enzymatic degradation.

It cannot be excluded that a [^{11}C]-labeled metabolite of **1** crosses the blood-brain barrier, and if such a metabolite has

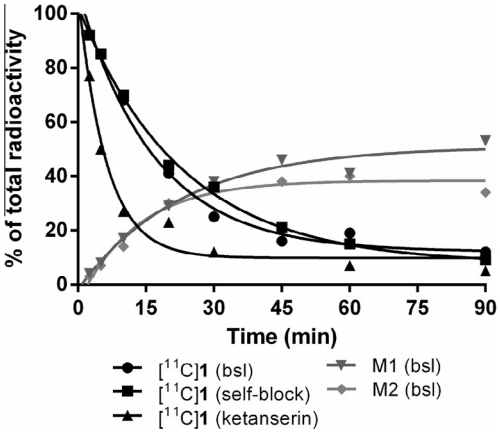


Figure 3. Rate of metabolism of [^{11}C]**1** (retention time = 6.3 min, black) in the indicated experiments and the accumulation of major metabolite (M1, retention time = 0.39 min, dark grey) and minor metabolite (M2, retention time = 4.9 min, light grey) at baseline.

affinity for a target with high density in the thalamus, this might explain the unexpected high binding seen in this region.

The lipophilicity of pimavanserin is relatively high with a calculated log*P* = 4.11 and the compound exhibits a volume of distribution in rats much larger than the total body water, indicating extensive partitioning into fatty tissues.[13] The high lipophilicity is presumably the cause of the high level of non-specific binding observed for [^{11}C]**1**.

In conclusion, **1** was ^{11}C-labeled in good radiochemical yield from a readily available precursor. The PET studies showed that [^{11}C]**1** has high brain permeability dominated by non-specific binding proven by no decrease in binding with either unlabeled **1** or ketanserin.

Acknowledgments

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16. *tert-Butyl 4-(1-(4-fluorobenzyl)-3-(4-isobutoxybenzyl)ureido)-piperidine-1-carboxylate (**7**):* (4-isobutoxyphenyl)methanamine (331 mg, 1.846 mmol)was dissolved in 10 mL of CH_2Cl_2 and slowly added to triphosgene (548 mg, 1.846 mmol) dissolved in 20 mL of CH_2Cl_2. 2 mL of Et_3N (14.35 mmol) was then added and the reaction was stirred for 30 min. *tert*-Butyl 4-((4-fluorobenzyl)amino)piperidine-1-carboxylate (569 mg, 1.847 mmol) was dissolved in 10 mL of CH_2Cl_2 and slowly added to the reaction. Stirring was continued for one hour. 50 mL of water was added to the reaction and the aqueous phase was extracted with 2 × 50 mL of CH_2Cl_2. The combined organic phases were evaporated in vacuo and purified by dry column vacuum chromatography using heptane to heptane/EtOAc 1:1. Yield: 828.9 mg, 81%. ^{1}H NMR ($CDCl_3$, 400 MHz) 1.00 (d, *J* = 6.60 Hz, 6H) 1.38–1.50 (m, 11H) 1.67–1.74 (m, 2H) 2.03 (dd, *J* = 13.08, 6.48 Hz, 1H) 2.75 (br s, 2H) 3.66 (d, *J* = 6.60 Hz, 2H) 4.09–4.19 (m, 2H) 4.25 (d, *J* = 5.38 Hz, 2H) 4.29 (s, 2H) 4.48 (br s, 1H) 4.53–4.59 (m, 1H) 6.76 (d, *J* = 8.56 Hz, 2H) 6.98 (dt, *J* = 8.50, 4.19 Hz, 4H) 7.17 (dd, *J* = 8.44, 5.26 Hz, 2H). ^{13}C NMR ($CDCl_3$, 400 MHz) 19.1, 22.7, 28.3, 28.4, 29.0, 30.2, 31.9, 44.4, 45.2, 52.7, 74.5, 79.6, 114.5, 115.7, 128.6, 131.1, 133.8, 154.6, 158.0, 158.5, 162.1, (d. *J* = 246 Hz). LC–MS (m+23): 536.2 *m/z*.
17. *1-(4-Fluorobenzyl)-3-(4-isobutoxybenzyl)-1-(piperidin-4-yl)urea (**8**): tert*-Butyl-4-(1-(4-fluorobenzyl)-3-(4-isobutoxybenzyl)-ureido)piperidine-1-carboxylate (248 mg, 0.483 mmol) was dissolved in 10% TFA:CH_2Cl_2 (10 mL) and stirred for 15 min. The reaction was added 8 mL of sat. $NaHCO_3$, 6 mL water and extracted with 2 × 20 mL CH_2Cl_2. Yield: 195.7 mg, 98%. ^{1}H NMR ($CDCl_3$, 400 MHz) 1.01 (d, *J* = 6.60 Hz, 6H) 1.50–1.65 (m, 2H) 1.76 (d, *J* = 10.03 Hz, 2H) 1.99–2.12 (m, 1H) 2.72 (t, *J* = 12.23 Hz, 2H) 3.13 (d, *J* = 12.23 Hz, 2H) 3.38 (br s, 1H) 3.68 (d, *J* = 6.60 Hz, 2H) 4.27 (d, *J* = 5.38 Hz, 2H) 4.35 (s, 2H) 4.38–4.47 (m, 1H) 4.51 (t, *J* = 5.26 Hz, 1H) 6.73–6.82 (m, 2H) 6.94–7.06 (m, 4H) 7.19 (dd, *J* = 8.31, 5.38 Hz, 2H). ^{13}C NMR ($CDCl_3$, 400 MHz) 19.2, 28.3, 30.8, 44.4, 45.2, 45.9, 52.6, 53.4, 74.5, 114.5, 115.9, 127.7, 128.6, 131.1, 134.0, 156.0, 158.5, 162.1, (d. *J* = 250 Hz LC–MS (m+1): 414.1 *m/z*.
18. *[^{11}C]-Labeling of pimavanserin (radiosynthesis of [^{11}C]**1**):* 0,3 mg of precursor was dissolved in 300 µl of acetone. After the trapping of [^{11}C]MeTf the reaction was heated to 60 °C for 90 s. Purification was performed by semi-preparative HPLC using an Onyx monolith C18 column (Phenomenex Inc.) (100 × 10 mm, 35:65 0.01 M Borax buffer/AcN, flowrate: 6 mL/min, retention time: 275–340 s). The collected fraction was trapped on a solid-phase C18 sep-pack light extraction column (waters) and eluted with 3 mL of EtOH. Results were analyzed by HPLC using a Luna 5 µm C18 100 Å column (Phenomenex Inc.) (150 × 4.6 mm, 35:65 0.01 M Borax buffer: can, flowrate: 2 mL/min, retention time: 3 min). Isolated yields: 1036–1743 MBq (*n* = 4). Specific activities: 34–80 GBq/µmol (*n* = 4). Radiochemical purity >98%, total synthesis time: 45 min. [^{11}C]Methane was produced via the ^{14}N(p,α) ^{11}C reaction by bombardment of a [^{14}N]N_2 containing 10% H_2 target with a 17 MeV proton beam in a Scanditronix MC32NI cyclotron. Starting activity around 100 GBq EOB as [^{11}C]methane. [^{11}C]Methane was converted into [^{11}C]MeI by the gas phase method using I_2 at 720 °C and subsequently converted into [^{11}C]MeOTf using silver triflate at 200 °C.
19. *PET data acquisition:* Two female Danish Landrace pigs (18 and 24.2 kg) were used in this study. The Danish Council for Animal Ethics approved the animal procedures (journal no. 2012-15-2934-00156). The animals were housed under standard conditions and were allowed to acclimatize for 1 week. Before scanning, the pigs were tranquilized with midazolam (0.5 mg/kg intramuscular (im)) and anaesthesia was induced with im injection of 1 mL/kg Zoletil veterinary mixture (6.25 Pt. xylazine (20 mg/mL) + 1.25 Pt. ketamine (100 mg/mL) + 2 Pt. butorphanol (10 mg/mL) + 2 Pt. methadone (10 mg/mL); Virbac, Kolding, Denmark). Hereafter, anaesthesia was maintained with constant propofol infusion (10 mg/kg/h intravenous (iv); B. Braun, Melsungen, Germany). Venous access was granted through two catheters in the peripheral milk veins. During anaesthesia, animals were endotracheally intubated and ventilated. Vital parameters (heart rate, body temperature, blood pressure, oxygen saturation and end tidal CO_2) were continuously monitored during the scan. The pigs were euthanized immediately after scanning with an iv injection of pentobarbital. [^{11}C]**1** was given as an intravenously (iv) bolus injection and the injected dose were 469 and 490 MBq (in 4.5 mL and 7 mL) for the baseline scan and 485 and 460 MBq (in 7.5 mL and 6 mL) for the ketanserin pre-treatment scan. The pig was subsequently scanned for 90 min in list-mode with a high resolution research tomography (HRRT) scanner (Siemens AG, Munich, Germany), where scanning started at the time of injection (0 min). Immediately after the end of the first scan the pig is treated with an iv bolus injection of 1 mg/kg ketanserin (Sigma–Aldrich) followed by continuous infusion of 1 mg/kg/h ketanserin for the duration of the second scan. The administration of ketanserin was given 21 min prior to the second injection of [^{11}C]**1**. For the self-block experiment, 6.4 mg pimavanserin was co-administrated with the radioligand. The dose was based on half-maximum reduction in binding in occupancy PET-study by Nordstrøm et al. (*International Journal of Neuropscyho-pharmacology*, **2008**, *11*, 163). The human dose (5 mg) was calculated to a pig dose (6.4 mg or 0.26 mg/kg) based on body surface area (Kelley et al., *Journal of Animal Science*, **1973**, *36*, 927).
Image reconstruction and analysis: Ninety-minute list-mode PET data were reconstructed into 38 dynamic frames of increasing length (6 × 10, 6 × 20, 4 × 30, 9 × 60, 2 × 180, 8 × 300, and 3 × 600 s). Images consisted of 207 planes of 256 × 256 voxels of 1.22 × 1.22 × 1.22 mm. A summed picture of all counts

in the 90-min scan was reconstructed and used for co-registration to a standardized MRI-based atlas of the Danish Landrace pig brain. The time-activity curve was calculated for the following volumes of interest (VOIs): cerebellum, cortex, lateral and medial thalamus. The radioactivity in thalamus is calculated as the mean radioactivity in the lateral and medial thalamus. Radioactivity in all VOIs was calculated as the average of radioactive concentration (Bq/mL) in the left and right sides. Outcome measure in the time-activity curves (TACs) was calculated as radioactive concentration in VOI (in kBq/mL) normalized to the injected dose corrected for animal weight (in kBq/kg), yielding standardized uptake values (g/mL).

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22. *Metabolite analysis:* The fraction of unchanged [^{11}C]**1** was determined by direct injection of plasma into a radio-HPLC system (Dionex Ultimate 3000) configured for column switching. Manually drawn arterial whole blood samples were centrifuged (1500×*g*, 7 min, 4 °C), and plasma was filtered through a syringe filter (Whatman GD/X 13 mm or 25 mm, PVDF membrane, 0.45 μm pore size) prior to the analysis by HPLC. To increase sensitivity on gamma counts from samples with low levels of radioactivity, eluent from the HPLC was collected into fractions (10 mL) using a fraction collector (Foxy Jr FC144; Teledyne) and counted offline in a well counter (2480 Wizard2 Automatic Gamma Counter, Wallac Oy). Representative HPLC-trace.