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^{11}C-labeling and preliminary evaluation of vortioxetine as a PET radioligand

Valdemar L. Andersen [a,b], Hanne D. Hansen [a], Matthias M. Herth [a], Gitte M. Knudsen [a], Jesper L. Kristensen [a,b,*]

[a] Center for Integrated Molecular Brain Imaging (CIMBI), Rigshospitalet and University of Copenhagen, Blegdamsvej 9, 2100 Copenhagen, Denmark
[b] Department of Drug Design and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen, Universitetsparken 2, 2100 Copenhagen, Denmark

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ABSTRACT

Vortioxetine is a new multi-modal drug against major depressive disorder with high affinity for a range of different serotonergic targets in the CNS. We report the ^{11}C-labeling of vortioxetine with [^{11}C]MeI using a Suzuki-protocol that allows for the presence of an unprotected amine. Preliminary evaluation of [^{11}C]vortioxetine in a Danish Landrace pig showed rapid brain uptake and brain distribution in accordance with the pharmacological profile, all though an unexpected high binding in cerebellum was also observed. [^{11}C]vortioxetine displayed slow tracer kinetics with peak uptake after 60 min and with limited wash-out from the brain. Further studies are needed but this radioligand may prove to be a valuable tool in unraveling the clinical effects of vortioxetine.

Major depressive disorder (MDD) is a prevalent disease that is considered by the WHO to be one of the leading causes of disability worldwide.[1] MDD has traditionally been treated with tricyclic anti-depressants (TCAs) monoamine oxidase inhibitors (MAOIs), selective serotonin reuptake inhibitors (SSRIs) and serotonin–norepinephrine reuptake inhibitors (SNRI's).[2]

Recently, a new drug for the treatment of MDD made its way to the market. Vortioxetine (**1**) is a multi-modal acting drug with high affinity for a range of serotonergic targets, see Figure 1.[3] The antidepressant effects of **1** are thought to be mediated through three serotonergic targets: (1) inhibition of the 5-HTT which leads to an increase in extracellular 5-HT levels in the brain (in analogy to previous antidepressants); (2) agonism of 5-HT$_{1A}$R, which is believed to shorten the time to onset of clinical effects,[4] and (3) antagonism of 5-HT$_3$R. Preclinical studies indicate that antagonism of 5-HT$_3$R (among many other effects) could have positive effects on mood and cognitive dysfunction in patients with depression.[5]

The recommended starting dose of **1** is 10 mg per day, and PET studies in humans have shown that the occupancy at the 5-HTT is ~50% at 5 mg whereas the 5-HT$_{1A}$R occupancy as measured in a small sample was not measurable.[6] The occupancy at other 5-HTRs is currently unknown and the exact mode of action of **1** remains to be fully elucidated. Here, we set out to develop a ^{11}C-labeled version of **1** for PET studies. Access to such a radiotracer would open the possibility for conducting studies in different patient groups who respond differently to treatment, in analogy to previous studies with [^{11}C]Clozapine.[12] Based on the in vitro receptor affinities reported for **1** one would expect to see binding in thalamus,[7,8] cortex,[9] hippocampus[9,10] and striatum.[8,11]

The commonly employed ^{11}C-labeling strategy of O-, N- or S-methylation is not an option for labeling vortioxetine, whereas the aromatic methyl-groups are possible sites for labeling via a Suzuki-coupling with a suitable boronic acid derivate.[13] In theory, two different labeling sites are possible: the *ortho*- and the *para*-position. We chose to focus on the 2-position, as the required precursor would be readily available.

As **1** contains a secondary amine, one could envisage two labeling strategies, see Scheme 1: in route A, a precursor with a suitable protecting group (Pg) on the secondary amine is first ^{11}C-labeled and the Pg is subsequently removed. This would appear to be the safe option as labeling of the precursor using route B (in which the two steps are reversed) in principle could lead to labeling at the secondary nitrogen instead. The disadvantage with route A is that it requires a subsequent deprotection step after the ^{11}C-labeling, with concurrent loss of radiochemical yield, whereas Route B gives [^{11}C]**1** directly. We have previously developed procedures for the Pd-mediated ^{11}C-labeling of aryl boronic derivatives containing unprotected amines[14] and decided to try and developed a radio synthesis based on route B.

* Corresponding author. Tel.: +45 35336487; fax: +45 35306040.
E-mail address: jesper.kristensen@sund.ku.dk (J.L. Kristensen).

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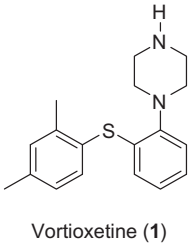


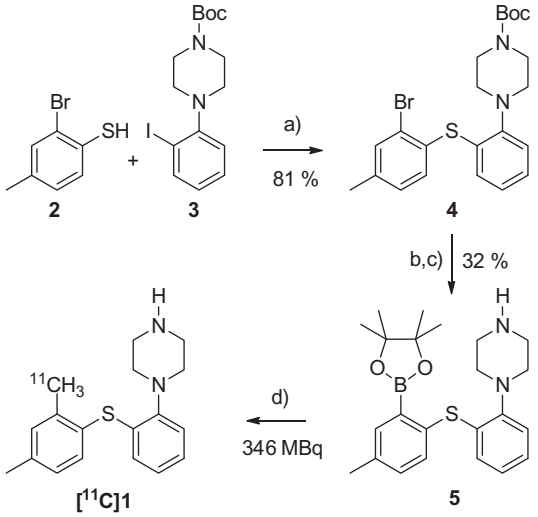
Target	Mode of action	Affinity (K_i, nM)
5-HTT	inhibitor	1.6
5-HT_{1A}	agonist	15
5-HT_{1B}	partial agonist	33
5-HT_{3A}	antagonist	3.7
5-HT_{7}	antagonist	19

Figure 1. Structure and selected pharmacological profile of vortioxetine (**1**) at various human 5-HT receptors.[3]

The required precursor was synthesized as outlined in Scheme 2. Starting from 2-bromo-4-methylbenzenethiol (**2**) and *tert*-butyl 4-(2-iodophenyl)piperazine-1-carboxylate (**3**) the synthesis of the required precursor was carried out in a three step sequence starting with a Palladium catalysed thioether formation via coupling of **2** and **3** to give **4** in 81% isolated yield.[15] The use of dry, degassed toluene and microwave irradiation at 100 °C proved essential for an efficient coupling; otherwise debrominated **4** was the main product. Installment of a boronic ester moiety via a Pd-catalyzed coupling with bis(pinacolato)diboron and $Pd(dppf)Cl_2$ followed by liberation of the secondary amine via Boc-deprotection provided the desired precursor **5** in 32% yield.[16]

We previously showed that by trapping $[^{11}C]CH_3I$ as a $[^{11}C]CH_3\text{–}PdL_n\text{-I}$ complex, it is possible to subsequently label aryl boronic derivatives that contain free amines.[14] Using this method **5** was converted to $[^{11}C]$**1** in 40% radiochemical yield. Following preparative HPLC-purification it was possible to isolate 346 MBq $[^{11}C]$**1** in >98% radiochemical purity and a specific activity of 478 GBq/μmol.[17] Yields were found to be extremely dependent on the quality of the palladium/ligand catalyst, as N-methylation rather than cross-coupling occurred in several instances, see Figure 2. Thus, a freshly prepared catalyst mixture was found to be essential for successful synthesis of $[^{11}C]$**1**.

$[^{11}C]$**1** was injected into a Danish Landrace pig (Fig. 3).[18] A swift brain uptake was observed and, as expected from the pharmacological profile, with high binding in cortex, thalamus, hippocampus, striatum. High binding in cerebellum was also observed which is unexpected as none of the identified targets are reported as having high densities in this part of the brain. Target densities have been determined in the pig brain with autoradiography for the 5-HTT ($[^3H]$escitalopram),[19] the 5-HT_{1A}R ($[^3H]$WAY-100635),[19] the 5-HT_{7}R ($[^3H]$SB-269970),[14b] and for the 5-HT_{3}R

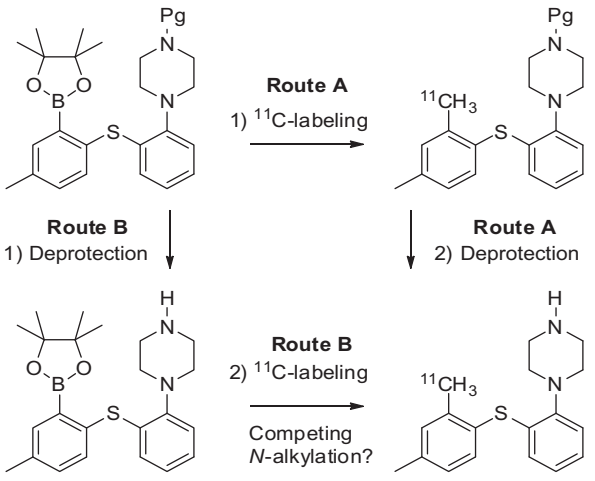


Scheme 2. Synthesis of precursor and ^{11}C-labeling of vortioxetine (**1**). Reagents and conditions: (a) Pd_2dba_3, DPEphos, *t*-BuOK, toluene, 100 °C (MW), 30 min; (b) bis(pinacolato)diboron, $Pd(dppf)Cl_2$, KOAc, 1,4-dioxane, 100 °C, 12 h; (c) TFA, CH_2Cl_2; (d) $[^{11}C]CH_3I$, Pd_2dba_3, P(*o*-tolyl)$_3$, K_2CO_3, 60 °C DMF/H_2O 9:1, 5 min.

($[^3H]$-(*S*)-Zacopride).[20] Areas with high density of 5-HT_{1A}R include the cortex and the hippocampus. 5-HT_{7}R density is high in the thalamus and in the cortex, but based on the affinity of **1** for these two targets it is most likely that the uptake of $[^{11}C]$**1** in the cortex arises from $[^{11}C]$**1** binding to the 5-HT_{1A}R.[21]

The high uptake in the striatum is likely due to the high density of 5-HTT and possibly also due to binding to the 5-HT_{1B}R. The area with the largest 5-HT_{3}R density in the pig brain is the spinal cord (substantia gelatinosa), however this region is too small to be identified on the PET image and the region is not available in the pig atlas. It is therefore difficult to analyze whether binding to the 5-HT_{3}R contributes to the PET signal.

The uniform uptake of $[^{11}C]$**1** could also indicate that the compound is very lipophilic and therefore has a large fraction of non-specific binding in the brain. The radio ligand displayed slow tracer kinetics with peak uptake after 60 min and with limited wash-out from the brain within the data acquisition time. This complicates the use of compartment models when performing kinetic modeling for quantification of binding, however in humans it may be possible to use reference tissue models although this needs to be validated.



Scheme 1. Possible radiolabeling approaches to ^{11}C-labeling of vortioxetine.

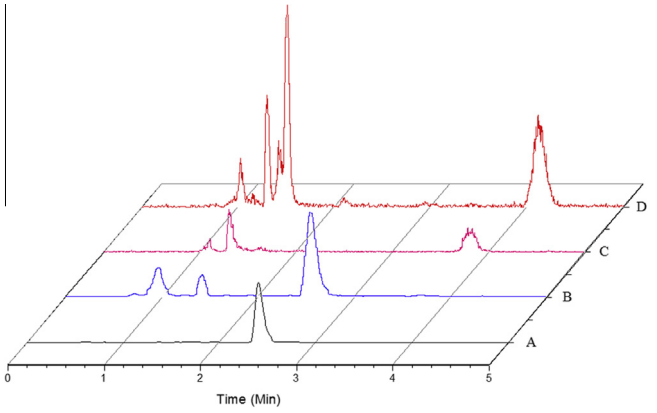


Figure 2. Effect of the state of the palladium–ligand combination on the amount of N-methylation. (A) Reference spectrum of **1** (with UV-detection). (B) Successful ^{11}C-labeling of **1**. (C) Unsuccessful ^{11}C-labeling of **1**—product with retention time ~4 min presumably the N-methylated precursor. (D) No Pd catalyst added—product with retention time ~4 min presumably the N-methylated precursor.

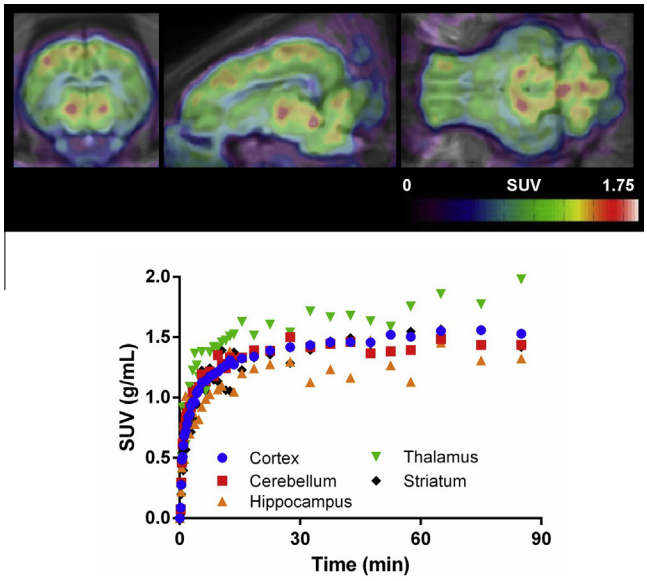


Figure 3. *Top:* Coronal, sagittal and transverse (left to right) summed PET images (0–90 min, 3 mm Gaussian filtering) of [^{11}C]**1** in the pig brain. *Bottom:* Time–activity curves showing absolute radioligand uptake for the indicated brain regions. SUV: standardized uptake value.

The protein binding of [^{11}C]**1** in pig plasma was determined to 98% measured after 2.5 h dialysis.[22] This level of protein binding is similar to that found in humans.[3]

In conclusion, vortioxetine was ^{11}C-labeled using the Suzuki reaction and its kinetics was evaluated in the pig brain. The tracer crossed the blood-brain barrier readily and binding to multiple sites within the brain was observed. Further investigations are necessary, but [^{11}C]vortioxetine could prove to be an important tool making it is possible to follow the pharmacokinetics of an antidepressant with a new mechanism of action and thus aid uncovering of the underlying mechanism for **1**'s effect on MDD.

Acknowledgments

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15. *Experimental section—synthesis of precursor: tert-butyl 4-(2-((2-bromo-4-methylphenyl)thio)phenyl)piperazine-1-carboxylate (**4**):* *tert*-BuOK (311 mg, 2.78 mmol), Pd_2dba_3 (57.8 mg, 0.063 mmol), DPEphos (136 mg, 0.252 mmol), 2-bromo-4-methylbenzenethiol (512 mg, 2.52 mmol) and *tert*-butyl 4-(2-iodophenyl)piperazine-1-carboxylate (980 mg, 2.52 mmol) was dissolved in dry toluene (4 mL), kept under nitrogen and degassed using a stream of nitrogen for 10 min. The mixture was then heated to 100 °C (MW). The resulting crude was purified by dry column vacuum chromatography using heptane to heptane/EtOAc 10:1 (rf = 0.48 heptane/EtOAc 3:1) yielding 950 mg of **4** as a slightly yellow oil (81%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.49 (s, 9H) 2.35 (s, 3H) 2.97–3.03 (m, 4 H) 3.50–3.55 (m, 4 H) 6.85 (dd, J = 7.78, 1.51 Hz, 1H) 6.95–6.99 (m, 1H) 7.04–7.08 (m, 2H) 7.17–7.23 (m, 2H) 7.51 (d, J = 1.00 Hz, 1H). ^{13}C NMR ($CDCl_3$, 400 MHz) δ: 20.8, 28.5, 51.6, 79.7, 120.4, 124.6, 127.3, 127.7, 129.0, 129.6, 131.7, 132.1, 134.0, 134.2, 139.6, 150.4, 154.9. LC–MS (m+1): 464 *m/z*.
16. *1-(2-((4-Methyl-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)thio)phenyl)piperazine (**5**):* Pd(dppf)Cl_2 (13.3 mg, 0.018 mmol), bis(pinacolato)diboron (169 mg, 0.665 mmol), KOAc (178 mg, 1.81 mmol) and 4 (280 mg, 0.604 mmol) was dissolved in dry 1,4-dioxane (10 mL), degassed with nitrogen for 10 min and heated to 100 °C for 18 h. The resulting crude was purified by dry column vacuum chromatography using heptane to heptane/EtOAc 10:1 (rf = 0.55 heptane/EtOAc 3:1). Yielding 124 mg of Boc-protected **5** as a slightly yellow oil (40%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.15 (s, 12H) 1.50 (s, 9H) 2.38 (s, 3H) 3.05 (br s, 4 H) 3.58 (br s, 4H) 6.74 (d, J = 7.83 Hz, 1H) 6.88 (t, J = 7.46 Hz, 1H) 7.00 (m, 1H) 7.06 (m, 1H) 7.22 (d, J = 7.82 Hz, 1H) 7.35 (d, J = 7.83 Hz, 1H) 7.63 (s, 1H). ^{13}C NMR ($CDCl_3$, 400 MHz) δ: 21.1, 24.6, 28.5 51.4, 79.6, 83.8, 119.5, 124.5, 125.6, 127.2, 132.3, 134.6, 134.8, 136.4, 137.1, 137.4, 155.0. LC–MS (m+1): 511 *m/z*. This material was Boc-deprotected in the following way: (90 mg, 0,176 mmol) was dissolved in CH_2Cl_2 (10 mL) and TFA (0.5 mL) was added and the mixture was stirred for 30 min. The resulting mixture was then washed with saturated aqueous $NaHCO_3$ (3 × 10 mL) and the organic phase was evaporated to dryness yielding 57 mg of **5** as a slightly yellow oil, 0,14 mmol, 79%) 1H NMR ($CDCl_3$, 400 MHz) δ: 1.13 (s, 10H) 2.38 (s, 3H) 3.37 (d, J = 12.23 Hz, 8H) 6.77 (d, J = 7.58 Hz, 1H) 6.93 (s, 1H) 7.02–7.05 (m, 1H) 7.09 (d, J = 7.09 Hz, 1H) 7.23 (d, J = 6.36 Hz, 1H) 7.34 (d, J = 7.82 Hz, 1H) 7.66 (s, 1H). ^{13}C NMR ($CDCl_3$, 400 MHz) δ: 21.0, 24.5, 44.2, 48.3, 83.9, 119.8, 125.5, 125.9, 127.4, 132.5, 134.1, 134.7, 136.5, 137.6, 147.2. LC-MS (m+1): 411 *m/z*.
17. ^{11}C-labeling of **1**: **5**, $Pd_2(dba)_3$, P(*o*-tolyl)$_3$ and base were used in a ratio of 40:1:2:4 with appropriate masses calculated from the use of 0.1 mg of P(*o*-tolyl)$_3$. [^{11}C]MeI was trapped in 300 μL DMF. To the trapped [^{11}C]MeI was added Pd-catalyst and K_2CO_3 dissolved in 300 μL of DMF/water 9:1 and heated to 60 °C. [^{11}C]MeI was allowed to react for 2 min. 5 was added dissolved in 150 μL of DMF/water 9:1 and allowed to react for 5 min. Purification was performed by preparative HPLC using a Luna 5 μm C18(2) 100 Å column (Phenomenex Inc.) (250 × 10 mm, 50:50 citrate buffer pH 4.62: AcN, flowrate: 9 mL/min). The collected fraction was trapped on a solid-phase C18 sep-pack extraction column and eluted with 3 ml EtOH. Results were analyzed by HPLC using a Luna 5 μm C18 100 Å column (Phenomenex Inc.) (150 × 4.6 mm. 50:50 citrate buffer pH 4.62: AcN, flowrate 2 mL/min). Starting activities around 100 GBq EOB as [^{11}C]CH_4. Yields as determined by HPLC: 40%. Isolated yields: 161.4–346 MBq. Specific activities: 8–478 GBq/μmol. Total synthesis time: 55 min.
18. *PET data acquisition:* A female Danish Landrace pig (18 kg) was tranquilized by intramuscular (im) injection of 0.5 mg/kg midazolam. Anaesthesia was induced by im injection of a Zoletil veterinary mixture (1.25 mg/kg tiletamin, 1.25 mg/kg zolazepam, and 0.5 mg/kg midazolam; Virbac Animal Health, France). Following induction, anaesthesia was maintained by intravenous (iv) infusion of 15 mg/kg/h propofol (B. Braun Melsugen AG). During anaesthesia the pig was endotracheally intubated and ventilated (volume 200 mL, frequency 16 per min). Venous access was granted through two catheters in the peripheral milk veins. The Danish Council for Animal Ethics approved the animal procedures (journal no. 2012-15-2934-00156). [^{11}C]**1** was given as an intravenously (iv) bolus injection and the injected dose was 124 MBq (n = 1). 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). 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, hippocampus, lateral and medial thalamus, caudate nucleus, and putamen. Striatum is defined as the mean radioactivity in caudate nucleus and putamen. 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. Plasma protein binding assay: The free fraction of [^{11}C]**1** in plasma, f_p, was estimated using an equilibrium dialysis chamber method. The dialysis was conducted in chambers (Harvard Biosciences) separated by cellulose membrane with a protein cut-off of 10,000 Da. Small amounts of [^{11}C]**1** (~5 MBq) were added to 5 mL plasma sample from the pig. Plasma (500 μL) was then dialysed at 37 °C against an equal volume of buffer (135 mM NaCl, 3.0 mM KCl, 1.2 mM $CaCl_2$, 1.0 mM $MgCl_2$, and 2.0 mM KH_2PO_4, pH 7.4). Counts per minute in 400 μL of plasma and buffer were determined in a well counter after various dialysis times (0.5, 1, 2, and 2.5 h), and f_p of [^{11}C]**1** was calculated as the ratio of radioactivity in buffer and plasma. Equilibrium in f_p was reached after 1 h of dialysis.