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Synthesis and evaluation of two new candidate high-affinity full agonist PET radioligands for imaging 5-HT_{1B} receptors

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Abstract

Introduction: The serotonin **1B** receptor subtype is of interest in the pathophysiology and treatment of depression, anxiety, and migraine. Over recent years 5-HT_{1B} receptor binding in human brain has been examined with PET using radio ligands that are partial but not full agonists. To explore how the intrinsic activity of a PET radio ligand may affect imaging performance, two high-affinity full 5-HT_{1B} receptor agonists (AZ11136118, **4**; and AZ11895987, **5**) were selected from a large compound library and radiolabeled for PET examination in non-human primates.

Methods: [¹¹C]**4** was obtained through Pd(O)-mediated insertion of [¹¹C]carbon monoxide between prepared iodoarene and homochiral amine precursors. [¹¹C]**5** was obtained through *N*-¹¹C-methylation of *N*-desmethyl precursor **6** with [¹¹C] methyl triflate. [¹¹C]**4** and [¹¹C]**5** were studied with PET in rhesus or cynomolgus monkey. [¹¹C]**4** was studied with PET in mice and rats to measure brain uptake and specific binding. Ex-vivo experiments in rats were performed to identify whether there were radiometabolites in brain. Physicochemical parameters for [¹¹C]**4** (p*K*_a, log*D* and conformational energetics) were evaluated.

Results: Both [¹¹C]**4** and [¹¹C]**5** were successfully produced in high radiochemical purity and in adequate amounts for PET experiments. After intravenous injection of [¹¹C]**4**, brain radioactivity

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.nucmedbio.2019.01.005>.

peaked at a low level (0.2 SUV). Pretreatment with tariquidar, an inhibitor of the brain P-gp efflux transporter, increased brain exposure four-fold whereas pretreatment with a high pharmacological dose of the 5-HT_{1B} antagonist, AR-A000002, had no effect on the binding. Ex-vivo experiments in rats showed no radiometabolites entering brain. [¹¹C]5 also failed to enter monkey brain under baseline conditions.

Conclusions: [¹¹C]4 and [¹¹C]5 show too low brain uptake and specific binding to be useful PET radio ligands. Low brain uptake is partly ascribed to efflux transporter action as well as unfavorable conformations.

Keywords

Serotonin subtype **1B** receptor; Radioligand; Agonist; Carbon-11; Carbonylation

1. Introduction

The brain serotonin subtype 1B (S-HT_{1B}) receptor is implicated in depression, anxiety, and migraine [1]. Therefore, there is interest to study this receptor in living subjects with positron emission tomography (PET) to gain insight into neuropsychiatric disorders. PET with suitable radioligands might also assist in developing drugs targeting the 5-HT_{1B} receptor [2]. Effective radioligands, labeled with positron-emitting carbon-11 ($t_{1/2} = 20.4$ min) or fluorine-18 ($t_{1/2} = 109.8$ min), are therefore required for imaging and quantifying these receptors in living human brain with PET [3].

The 5-HT_{1B} receptor is a G-protein coupled receptor (GPCR) that is expressed on axons for serotonin throughout the central nervous system (CNS) [4]. Receptor concentrations are high in occipital cortex and globus pallidus, and negligible in cerebellum. The G-protein is negatively coupled to adenylate cyclase such that when the 5-HT_{1B} receptor is activated the cyclase activity is depressed. 5-HT_{1B} receptors serve as presynaptic autoreceptors and postsynaptic heteroreceptors [5]. 5-HT_{1B} autoreceptors serve to control release of serotonin into the synaptic cleft. Agonists inhibit release of serotonin whereas antagonists promote release. 5-HT_{1B} heteroreceptors play a regulatory role in the release of other neurotransmitters, like dopamine [6,7].

According to a long-standing two-state model, GPCRs generally exist in either of two affinity states for endogenous ligand, namely G-protein-coupled and G-protein-uncoupled, with these two states in dynamic equilibrium. How this equilibrium affects the binding of exogenous agonists or antagonists to GPCRs in vivo is not precisely known [8]. According to the two-state model, the binding of 5-HT_{1B} receptor agonists is expected to be more sensitive to G-protein coupling than that of antagonists, with agonists likely binding only to the high affinity state and antagonists to both states [9]. However, an increasing body of evidence indicates that this model is insufficient, and that multiple conformational states of GPCRs can be involved in the binding of ligands [10,11].

The few effective PET radioligands ([¹¹C]1-[¹¹C]3) that have been reported so far for imaging 5-HT_{1B} receptors are now considered to be full or partial antagonists (Fig. 1) [12–14]. The influence of the intrinsic activity of a PET radioligand on 5-HT_{1B} receptor imaging

performance has not been explored and therefore is not well understood [15]. An agonist is expected to have selectivity for binding to the G-protein coupled state of the 5-HT_{1B} receptor and to be more useful for studying physiologically or pharmacologically induced changes in synaptic serotonin levels.

In the present study, we aimed to develop a full agonist PET radioligand for imaging brain 5-HT_{1B} receptors to compare with previously reported radioligands that are not full antagonists for their relative sensitivities to changes in synaptic serotonin levels [16]. From an AstraZeneca library of over 3000 high-affinity 5-HT_{1B} receptor ligands, we identified AZ11136118 (K_i ; = 0.25 nM, EC_{50} < 0.17 nM, agonist GTP γ S efficacy 112%; **4**) and AZ11895987 (K_i ; = 0.46 nM, EC_{50} < 0.98 nM, agonist GTP γ S efficacy 113%; **5**) as high-affinity full agonists with other favorable properties for PET radioligand development, including amenability for labeling with a positron-emitter and properties expected to be conducive to good brain entry (**4**, clog P 3.0, PSA 81 Å²; **5**, clog P 3.1, PSA 45 Å²) [17]. Here we report the syntheses of [¹¹C]**4** and [¹¹C]**5** (Fig. 1) and their evaluations as candidate PET radioligands in rodents and monkey.

2. Materials and methods

2.1. General

All work in this study involving [¹¹C]**4** performed at the Molecular Imaging Branch, NIMH, Bethesda, MD, USA

All work in this study involving [¹¹C]**5** was performed at the PET centre, Karolinska Institutet, Stockholm, Sweden.

¹H (400.13 MHz) and ¹³C (100.62 MHz) NMR spectra were recorded on an Avance 400 instrument (Bruker) at room temperature in deuterated solvents. NMR signals are reported as δ (ppm) downfield from the signal of tetramethylsilane. HRMS data (ESI-TOF) were obtained at the Bioorganic Chemistry Laboratory of NIDDK (NIH). Optical rotations were measured on a P-1 010 polarimeter (JASCO).

2.2. Chemistry

2.2.1. N-Benzyl-8-methoxy-1,2,3,4-tetrahydronaphthalen-2-amine (8)—8-Methoxy-tetralone (**7**, 2.5 g, 14 mmol) was taken up in methanol (100 mL) at 0 °C and benzylamine (1.5 g, 14 mmol) and acetic acid (1.5 mL) were added. The mixture was stirred on ice for 30 min before addition of sodium cyanoborohydride (1.3 g, 21 mmol) and then allowed to reach ambient temperature overnight. Water (50 mL) was added and crude product was extracted into ethyl acetate (100 mL $\times$ 3) and dried (Na₂SO₄). Product was isolated with flash column chromatography (silica gel; 0–10% methanol in DCM). Desired fractions were pooled and concentrated in vacuo to give **8** as a white solid (3.2 g, 12 mmol, 85%). ¹H NMR (400 MHz, CDCl₃) δ 7.53 (*d*, *J* = 7.4 Hz, 2H), 7.34 (m, 3H), 7.10 (*t*, *J* = 7.9 Hz, 1H), 6.65 (*t*, *J* = 7.3 Hz, 2H), 4.23 (*d*, *J* = 13.2 Hz, 1H), 4.12 (*d*, *J* = 13.2 Hz, 1H), 3.75 (s, 3H), 3.26 (m, 2H), 2.77 (m, 3H), 2.27 (*t*, *J* = 6.1 Hz, 1H), 1.88 (m, 1H). ¹³C NMR (101 MHz, CDCl₃) δ 157.1, 135.8, 130.2, 129.8, 129.6, 129.3, 127.2, 120.7, 107.3, 55.2, 53.9,

48.4, 27.8, 25.9, 25.7, 18.3. HRMS (TOF ES+) m/z $[M + H]^+$ calculated for $C_{18}H_{22}NO$ 268.1701, found 268.1706.

2.2.2. 2-Isopropyl-5-methylcyclohexyl benzyl(8-methoxy-1,2,3,4-tetrahydronaphthalen-2-yl)carbamate (9)—Amine **8** (1.3 g, 4.8 mmol) was taken up in acetonitrile (10 mL). TEA (1.0 mL) and *R*-menthyl chloroformate (1.1 g, 5.0 mmol) were added sequentially. The reaction mixture was stirred for 2 h at ambient temperature and then concentrated in vacuo. The product was isolated with flash column chromatography (silica gel; 10–50% ethyl acetate in hexane). The desired fractions were pooled and concentrated to give **9** as a thick oil (2.0 g, 4.4 mmol, 92%). 1H NMR (400 MHz, $CDCl_3$) δ 7.27 (m, 5H), 7.08 (*t*, J = 7.8 Hz, 1H), 6.66 (*dd*, J = 8.1, 11.4 Hz, 2H), 4.39 (*t*, J = 86.0 Hz, 4H), 3.79 (s, 3H), 3.01 (s, 1H), 2.82 (s, 1H), 2.61 (m, 1H), 2.10 (d, J = 10.6 Hz, 1H), 1.80 (q, J = 9.3 Hz, 2H), 1.61 (d, 3H), 1.48 (s, 1H), 1.14 (m, 3H), 0.89 (*d*, J = 6.1 Hz, 6H), 0.80 (s, 7H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.4, 140.1, 137.0, 1283, 126.7, 126.3, 124.4, 120.8, 106.9, 75.2, 55.2, 47.2, 46.8, 41.5, 41.4, 34.3, 31.4, 29.9, 26.3, 23.4, 23.1, 22.1, 20.9, 16.3, 16.1. HRMS (TOF ES+) m/z $[M + H]^+$ calculated for $C_{29}H_{40}NO_3$ 450.3008, found 450.3012.

2.2.3. 2-Isopropyl-5-methylcyclohexyl benzyl(8-hydroxy-1,2,3,4-tetrahydronaphthalen-2-yl)carbamate (10)—**9** (0.5 g, 1.1 mmol) was taken up in anhydrous DCM (5 mL) under argon and cooled to between $-30^\circ C$ and $-40^\circ C$. BBr_3 (1.0 M; 1.65 mL) was added. After 1 h, the mixture was allowed to reach ambient temperature and then stirred for another 2 h. The mixture was poured onto ice-water (100 mL) and then extracted with DCM (20 mL $\times$ 3). The organic layers were combined, dried (Na_2SO_4), and concentrated in vacuo. Product was isolated by flash column chromatography (silica gel 0–30% ethyl acetate in hexane). The desired fractions were pooled and concentrated to give **10** as a thick oil (0.4 g, 0.92 mmol, 83%). 1H NMR (400 MHz, $CDCl_3$) δ 7.27 (m, 5H), 6.91 (s, 1H), 6.61 (d, J = 7.6 Hz, 2H), 4.50 (m, 4H), 3.01 (s, 1H), 2.75 (s, 2H), 2.62 (*t*, J = 14.0 Hz, 1H), 2.11 (*t*, J = 5.5 Hz, 1H), 1.75 (m, 1H), 1.64 (*d*, J = 12.2 Hz, 3H), 1.47 (d, J = 3.1 Hz, 1H), 0.99 (m, 2H), 0.89 (d, J = 6.2 Hz, 6H), 0.76 (d, J = 15.7 Hz, 7H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.4, 154.0, 139.8, 127.1, 126.1, 122.4, 120.5, 112.0, 75.6, 60.5, 53.3, 47.1, 41.5, 34.3, 31.5, 22.7, 22.1, 21.1, 20.9, 14.2. HRMS (TOF ES+) m/z $[M + H]^+$ calculated for $C_{28}H_{38}NO_3$ 436.2852, found 436.2850.

2.2.4. 7-(Benzyl((2-isopropyl-5-methylcyclohexyloxy)carbonyl)amino)-5,6,7,8-tetrahydronaphthalen-1-yl trifluoromethanesulfonate (11)—**10** (0.4 g, 0.92 mmol) was taken up in DCM (10 mL) and cooled to $0^\circ C$. Pyridine (300 μ L) was added, followed by trifluoromethanesulfonic anhydride (310 mg, 1.1 mmol). The mixture was stirred at $0^\circ C$ for 10 min, warmed to ambient temperature and stirred for a further 1 h. The mixture was diluted with water (100 mL) and extracted with ethyl acetate (20 mL $\times$ 3). The organic layers were combined, dried (Na_2SO_4) and concentrated in vacuo. The product was isolated with flash column chromatography (silica gel; 0–20% ethyl acetate in hexane). The desired fractions were pooled and concentrated to give **11** as a thick oil (350 mg, 0.62 mmol, 68%). 1H NMR (400 MHz, $CDCl_3$) δ 7.32 (*d*, J = 7.7 Hz, 1H), 7.24 (m, 3H), 7.15 (*t*, J = 8.7 Hz, 2H), 7.06 (*d*, J = 7.0 Hz, 2H), 4.54 (*t*, J = 12.9 Hz, 4H), 3.02 (s, 1H), 2.86 (*d*, J = 17.1 Hz, 2H), 2.78 (*t*, J = 13.5 Hz, 1H), 2.11 (*d*, J = 10.8 Hz, 1H), 1.90 (s, 2H), 1.65 (*d*, J = 10.6 Hz, 2H), 1.49 (s,

1H), 1.26 (s, 1H), 1.04 (*d*, *J* = 8.7 Hz, 2H), 0.90 (d, *J* = 6.4 Hz, 6H), 0.80 (s, 7H). ¹³C NMR (101 MHz, CDCl₃) δ 148.1, 139.4, 139.2, 137.9, 129.1, 129.0, 128.6, 128.5, 128.3, 127.1, 126.7, 125.3, 120.2, 118.5, 117.0, 113.8, 75.6, 52.8, 47.4, 41.4, 34.3, 31.5, 29.8, 27.6, 27.3, 26.0, 23.2, 22.1, 21.5, 20.9, 16.1. HRMS (TOF ES+) *m/z* [*M* + Na]⁺ calculated for C₂₉H₃₆NO₅F₃SNa 590.2164, found 590.2156.

2.2.5. 2-Isopropyl-5-methylcyclohexyl-benzyl(8-(4-methylpiperazin-1-yl)-1,2,3,4-tetrahydronaphthalen-2-yl)carbamate (12)—11 (510 mg, 0.90 mmol) in toluene (2 mL) was added to a stirred solution of Pd₂dba₃ (25 mg), BINAP (40 mg) and Cs₂CO₃ (385 mg, 1.2 mmol) in toluene (5 mL) under argon. 1-Methylpiperazine (110 mg, 1.0 mmol) was added and the reaction was heated to 80 °C overnight. The mixture was then filtered through celite, concentrated, and purified with flash column chromatography (silica gel; 10% methanol in DCM) to give a mixture of the two diastereomers. The two diastereomers were subsequently isolated by flash column chromatography (silica gel; 3% methanol in DCM rising to 20% methanol only after the first diastereomer had completely eluted). Fractions for the two products were separately pooled and concentrated. No absolute configuration was determined. Diastereomer (*S*)-**12**, (230 mg, 0.41 mmol), diastereomer (*R*)-**12** (200 mg, 0.35 mmol) were both isolated as thick oils. The analytical data for diastereomer 2 are: ¹H NMR (400 MHz, CDCl₃) δ 7.31 (*d*, *J* = 6.3 Hz, 4H), 7.24 (*d*, *J* = 8.4 Hz, 1H), 7.09 (*t*, *J* = 7.6 Hz, 1H), 6.86 (*d*, *J* = 7.7 Hz, 1H), 6.82 (*d*, *J* = 7.6 Hz, 1H), 4.73 (s, 2H), 4.27 (d, *J* = 128.0 Hz, 2H), 2.98 (d, *J* = 15.4 Hz, 1H), 2.88 (s, 4H), 2.70 (s, 2H), 2.46 (*q*, *J* = 9.2 Hz, 4H), 2.34 (s, 3H), 2.13 (*t*, *J* = 15.1 Hz, 1H), 1.92 (s, 4H), 1.65 (s, 2H), 1.51 (s, 1H), 1.32 (*d*, *J* = 19.4 Hz, 1H), 1.06 (*d*, *J* = 8.1 Hz, 1H), 0.91 (*d*, *J* = 6.4 Hz, 6H), 0.81 (*d*, *J* = 25.6 Hz, 5H). ¹³C NMR (101 MHz, CDCl₃) δ 151.3, 140.2, 136.6, 131.0, 128.4, 127.2, 126.8, 126.3, 123.9, 116.8, 75.3, 55.6, 53.6, 51.6, 47.3, 46.1, 41.5, 34.3, 31.4, 30.0, 28.1, 26.2, 23.3, 22.1, 21.0, 16.4. HRMS (TOF ES+) *m/z* [*M* + H]⁺ calculated for C₃₃H₄₈N₃O₃ 518.3747, found 518.3757.

2.2.6. (R)-8-(4-Methylpiperazin-1-yl)-1,2,3,4-tetrahydronaphthalen-2-amine [(R)-13]—(*R*)-**12** (200 mg, 0.35 mmol) was mixed in a 1:2 solution (3 mL) of HBr (33 wt% in acetic acid) and formic acid in a sealed vessel. The reaction was heated to 80 °C overnight, neutralized to pH 7 with NaOH (6 M) and extracted with DCM (30 mL × 3). The organic layers were combined, dried and concentrated. The residue was taken up in methanol (5 mL) and Pd/C (10%, 30 mg) was added. The vessel was put under vacuum and flushed with argon twice before being pressurized with H₂ gas at 5 bar. The mixture was stirred overnight, filtered through celite, and concentrated to give (*R*)-**13** as an off-white solid (80 mg, 0.32 mmol). This product was determined to be the desired enantiomer by comparison of its specific optical rotation with that of reference sample of known pharmacological activity/absolute configuration. ¹H NMR (400 MHz, CDCl₃) δ 7.11 (*t*, *J* = 7.7 Hz, 1H), 6.91 (*d*, *J* = 7.6 Hz, 1H), 6.86 (d, *J* = 7.4 Hz, 1H), 3.15 (m, 2H), 3.03 (m, 2H), 2.91 (d, *J* = 4.5 Hz, 2H), 2.83 (s, 4H), 2.59 (s, 4H), 2.37 (s, 3H), 2.32 (*q*, *J* = 8.5 Hz, 1H), 2.13 (s, 4H), 2.00 (m, 1H), 1.60 (m, 1H). ¹³C NMR (101 MHz, CDCl₃) δ 170.2, 150.9, 136.0, 129.6, 125.2, 123.1, 115.8, 59.4, 52.3, 46.6, 45.5, 34.1, 31.7, 27.6, 20.0, 13.2. HRMS (TOF ES+) *m/z* [*M* + H]⁺ calculated for C₁₅H₂₄N₃ 246.1970, found 246.1969.

2.2.7. 4-Iodophenyl methanesulfonate (14)—4-Iodophenol (300 mg, 2.0 mmol) was taken up in THF (5 mL) and triethylamine (0.2 mL) was added followed by methanesulfonyl chloride (0.5 mL, 2.9 mmol). The mixture was stirred for 3 h, then extracted with DCM (20 mL $\times$ 3), dried (Na₂SO₄) and concentrated in vacuo. The product was isolated with flash chromatography (silica gel; 0–30% ethyl acetate in hexane). The desired fraction was pooled and concentrated in vacuo to give **14** as white solid (425 mg, quantitative yield). ¹H NMR (400 MHz, CDCl₃) δ 7.74 (*d*, *J* = 8.8 Hz, 2H), 7.05 (*d*, *J* = 8.9 Hz, 2H), 3.15 (s, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 148.9, 139.2, 124.1, 92.0, 37.6. HRMS (TOF ES+) *m/z* [*M* + H]⁺ calculated for C₇H₇O₃SI 298.9161, found 298.9158.

2.3. Pharmacological assays

Ligands **4** and **5** were assayed at AstraZeneca for 5-HT_{1B} receptors agonist and antagonist activity using a GTIγS SPA (96 well) protocol. Data were analyzed by calculating percent effect and/or IC₅₀ (antagonist assay; 50% of stimulated response) or EC₅₀ (agonist assay; half maximum of curve) using an activity base template (see Supporting Information for assay protocols).

2.4. Radiochemistry

2.4.1. Synthesis of [¹¹C]4****—[¹¹C]Carbon dioxide was produced with the ¹⁴N(p,α)¹¹C nuclear reaction in a target containing 1% oxygen in nitrogen, initially pressurized to 225 psi. This target was bombarded with a beam (45 μA) of protons (16.5 MeV) from a PETtrace cyclotron (GE Medical Systems; Milwaukee, WI). Irradiations were conducted for 40 min for radiotracer production and typically provided about 110 GBq of [¹¹C]carbon dioxide. Shorter irradiations were used to produce lower amounts of [¹¹C] carbon dioxide for initial radiochemistry experiments. At the end of irradiation, radioactivity was released to a trap containing molecular sieve (13×; 400 mg) at room temperature, which was then swept with helium at 80 mL min⁻¹ to remove residual oxygen.

[¹¹C]Carbon dioxide was released from the molecular sieve trap at 280 °C in a stream of helium (10 mL min⁻¹) for concentration in a trap containing silica (10 mg) cooled in liquid nitrogen. This trap was heated with a halogen lamp (150 W) to release [¹¹C]carbon dioxide in helium (10 mL min⁻¹) for conversion into [¹¹C]carbon monoxide by passage through a heated (875 °C) quartz tube (22 cm length; 0.7 cm i.d.) packed with molybdenum wire (99.97%, 0.05 mm diameter; Strem Chemicals, Newburyport, MA). The effluent was passed through ascarite to strip-off unconverted [¹¹C]carbon dioxide and the [¹¹C] carbon monoxide was then trapped in a second silica cryotrap at -196 °C in liquid nitrogen. By heating this trap with a halogen lamp, [¹¹C]carbon monoxide was released into helium for transfer to an autoclave preloaded with a solution of Pd₂dba₃ (1.3 mg, 1.4 μmol), Xantphos (3.0 mg, 5.2 μmol) and iodo-precursor (**14**; 2.4 mg, 8.1 μmol) in THF (80 μL). The autoclave was then pressurized to ~3500 psi with THF by a solvent pump (SSI Isocratic; Scantech Lab, Sweden) and heated at 150 °C for 3 min. The reaction mixture was then flushed out of the autoclave into a 5-mL glass vial (Alltech; Sigma Aldrich; StLouis, MI) that had been loaded with a solution of the R-amine [(*R*)-**13**], (3.2 mg, 13 μmol) and Bu₃N (10 μL, 42 μmol) in THF (50 μL). The vial was heated from 67 °C rising to 100 °C while THF was evaporated off under N₂ gas purge (200 mL min⁻¹; 3 min) through a vent needle.

The dry radioactive residue was re-dissolved in acetonitrile (700 μL) and diluted with water (2.5 mL). The aqueous mixture was injected onto a Luna C18 column (10 μm , 10 $\times$ 250 mm; Phenomenex; Torrance, CA) eluted at 6 mL min⁻¹ using a gradient of freshly prepared 0.2% aqueous NH₄OH (A, pH = 8.5) and acetonitrile (B) in the mobile phase. The gradient began at 10% B for 1 min, increased to 45% B over 25 min, and finally reached 90% B over 1 min. Absorbance was monitored at 254 nm (System Gold 166; Beckman Coulter Inc., Pasadena, CA) while radioactivity was monitored with a pin-diode detector (Bioscan Inc., Washington DC). The fraction eluting between 16.5 and 17.5 min was collected and then evacuated at 80 °C for 1 min to remove solvents. The radioactive residue was reconstituted in saline (9 mL) plus ethanol (1 mL) and sterilized by filtration through a 0.22 μm sterile filter (Millipore-MP; Waters Corp.; Milford, MA). An aliquot (~60 μL) of the formulated product was analyzed with radio-HPLC (high-performance liquid chromatography) on a Luna C18 column (10 μm , 4.6 $\times$ 250 mm; Phenomenex) to measure radiochemical purity and molar activity. The column was eluted at 2 mL min⁻¹ with a gradient of 0.2% aq. NH₄OH (A) and acetonitrile (B) as mobile phase. The gradient began at 40% B and was linearly increased to 70% B over 10 min. The eluate was monitored for absorbance at 254 nm (System Gold 166 detector) and for radioactivity with a PMT detector (Bioscan Inc.). The retention time of [¹¹C]**4** was 6.7 min. The absorbance response had been pre-calibrated with respect to mass of **4** in the injectate, to permit calculation of molar activity.

2.4.2. Synthesis of [¹¹C]5****—[¹¹C]Methane was prepared through the ¹⁴N(p, α)¹¹C reaction by bombarding a mixture of nitrogen and hydrogen (10%) gas with a beam of 16.7 MeV protons produced from a biomedical cyclotron (GE PETtrace; Uppsala, Sweden). [¹¹C]Methane was converted into [¹¹C]methyl iodide by high temperature iodination in a gas-phase recirculation system and subsequently transformed into [¹¹C]methyl triflate, as described previously [18,19]. [¹¹C]**5** was obtained by trapping [¹¹C] methyl triflate in a solution of *N*-desmethyl precursor **6** (0.1–0.3 mg) and sodium hydroxide (0.1 M, 6 μL) in acetone (400 μL) at room temperature. The sealed reaction vial was left for 2 min. The reaction mixture was injected onto a reversed phased HPLC column (ACE C-18; 250 $\times$ 10 mm; ACE) eluted with acetonitrile: HCO₂NH₄ (0.1 M) (35:65 v/v) at 5 mL min⁻¹. Eluate was monitored in series for absorbance at (254 nm) and radioactivity (Geiger-Muller tube). The fraction containing [¹¹C]**5** (t_R = 12–13.5 min) was collected, formulated in saline (pH = 6; 4–6 mL), and finally filtered through a sterile filter (0.22 μm : Millipore Millex®GV) into a sterile vial.

The radiochemical purity of the product was determined with reversed phase HPLC on ACE 5 C18-HL column (C18, 3.9 $\times$ 300 mm, 10 μm particle size; Advanced chromatography technologies Ltd), with eluate monitored in series for absorbance at 254 nm and radioactivity (β -flow detector; Beckman, Fullerton, CA). For the analysis, the column was eluted at 3 mL min⁻¹ with a gradient of 10–90% acetonitrile in HCO₂NH₄ (0.1 M) for 10 min. [¹¹C]**5** (t_R = 4.4 min) was identified by its co-mobility with reference sample of **5**.

The molar activity of the final product was measured with HPLC under the conditions described above for radiochemical analysis. The absorbance (λ = 254 nm) response was calibrated for mass of ligand and calculated as the radioactivity of the radio ligand (GBq)

divided by the amount of the associated carrier substance (μmol). Each sample was analyzed three times and compared to a reference standard also analyzed three times.

2.5. Apparent pK_a determination

The radiochemical purity of the $[^{11}\text{C}]\mathbf{4}$ preparation was 99.1% as determined by radio-HPLC on an X-Terra C_{18} column ($10\ \mu\text{m}$, $7.8 \times 300\ \text{mm}$; Waters Corp.) and elution with a mobile phase of $\text{MeOH}:\text{H}_2\text{O}:\text{Et}_3\text{N}$ (82.5:17.5:0.1 by vol.) at $4.0\ \text{mL min}^{-1}$.

Phosphate buffers (0.15 M) with pH 3.0 to 10.5 at half pH intervals were prepared from NaH_2PO_4 (0.15 M) and Na_2HPO_4 (0.15 M) and distributed 1 mL per tube. $[^{11}\text{C}]\mathbf{4}$ ($2.51 \pm 0.06\ \text{MBq}$) formulated in saline/10% ethanol containing 0.7 mM ascorbic acid ($5\ \mu\text{L}$, $n = 48$) was added to each test tube. Triple sets of such tubes were prepared simultaneously. Cyclohexane (1.0 mL) was added to each of the tubes containing the buffer and $[^{11}\text{C}]\mathbf{4}$. The tubes were vigorously vortexed for 1 min, and then centrifuged for 1 min. Each phase was then separated, sampled ($200\ \mu\text{L}$) and counted in a γ -counter. The distribution coefficient (D) was then calculated by dividing the counts detected in the organic phase by the total activity present in both phases.

The apparent pK_a value was determined as the pH value where the concentration of ionized and non-ionized $[^{11}\text{C}]\mathbf{4}$ were equal by plotting D versus pH and applying nonlinear regression analysis (GraphPad Prism version 4.03 for windows (GraphPad Software; San Diego, CA, USA) with “One site competition” curve-fitting).

2.6. LogD determination

The radioligand was placed in sodium phosphate buffer (0.15 M; pH 7.4) for the duration of the logD determination followed by the measurement of its radiochemical purity to obtain information on its stability in buffer. Furthermore, the radiochemical composition of the aqueous buffer remaining after their extraction with 1-octanol was determined with HPLC on an X-Terra C_{18} column ($10\ \mu\text{L}$, $7.8 \times 300\ \text{mm}$; Waters Corp.) and eluted with a mobile phase of $\text{MeOH}:\text{H}_2\text{O}:\text{Et}_3\text{N}$ (82.5:17.5:0.1 by vol.) at $3.5\ \text{mL min}^{-1}$. The HPLC system consisted of Beckman Gold analytic pumps (Beckman Coulter, Inc.), an in-line photodiode-array detector and a flow-through $\text{NaI}(\pi)$ scintillation detector-rate meter (Bioscan). Data from the radioanalysis were collected and stored with Bio-Chrome Lite software (Bioscan) and analyzed after decay correction of the radiochromatograms.

Sodium phosphate buffer (0.15 M) was prepared by mixing NaH_2PO_4 and Na_2HPO_4 solutions to give a buffer of 0.15 M concentration and pH of 7.4. 1-Octanol was pre-equilibrated overnight with phosphate buffer.

$[^{11}\text{C}]\mathbf{4}$ ($\sim 363\ \text{MBq}$ in $2.3\ \text{mL}$) was added to sodium phosphate buffer (0.15 M; $6.5\ \text{mL}$) and mixed well. Aliquots ($1\ \text{mL}$) were distributed to eight borosilicate disposable culture tubes ($13 \times 100\ \text{mm}$). Six tubes, each containing 1-octanol ($1.0\ \text{mL}$), and two tubes without 1-octanol, served for determining the stability of $[^{11}\text{C}]\mathbf{4}$ and adsorption to the walls of the tube by incubation for the duration of the study. Radioactivity from six tubes were extracted with 1-octanol by vortexing the tubes for 1 min. The tubes were then centrifuged for 1 min. The organic and the aqueous phases were separated. Each phase was then sampled ($50\ \mu\text{L}$ and

200 μL , respectively) and counted separately in a well-type γ -counter (model 1480 Wizard; Perkin-Elmer) with an electronic window set at between 360 and 1800 keV. The remaining two tubes contained the radioactivity in buffer and served as stability measures for the radioligand. Radioactivity in the aqueous phases (200 μL) resulted in counting errors of $0.7 \pm 0.03\%$ ($n = 6$) at one standard deviation. The aqueous phases were injected onto the HPLC. The resulting radiochromatograms provided correction factors for the γ -counter counts to determine parent radioactivity only (corrected radioactivity).

2.7. Monkey PET imaging using [^{11}C]4

PET imaging experiments in monkey at NIMH were performed in accordance with the Guide for Care and Use of Laboratory Animals and were approved by the National Institute of Mental Health Animal Care and Use Committee [20].

A single male rhesus monkey (*Macaca mulatta*; 11.7 kg) was used for four PET scan sessions in which the monkey was initially anesthetized with ketamine (10 mg kg^{-1} , i.m.) and then maintained in anesthesia with 1.5% isoflurane. Thus, at baseline [^{11}C]4 (306 MBq; 69 GBq μmol^{-1}) was injected intravenously as a bolus (session 1). On the same day, at >3 h later, AR-A000002 (2 mg kg^{-1} , i.v.) was administered intravenously at 15 min before [^{11}C]4 (343 MBq; 71 GBq μmol^{-1}) to pre-block 5-HT_{1B} receptors (session 2). For P-gp-preblocked conditions six weeks later, tariquidar (8 mg kg^{-1} , i.v.) was administered at 25 min before injection of [^{11}C]4 (292 MBq; 18 GBq μmol^{-1}) (session 3). Finally, 4 weeks later, the monkey was imaged after administration of [^{11}C]4 (240 MBq; 15 GBq μmol^{-1}) under both 5-HT_{1B}-receptor and P-gp pre blocked conditions achieved by giving tariquidar (8 mg kg^{-1} , i.v.) at 22 min before [^{11}C]4 and AR-A000002 (2 mg kg^{-1} , i.v.) at 15 min before [^{11}C]4 injection (session 4). Tariquidar treatments had to be at least four weeks apart for the same monkey for drug safety reasons. In each experiment, PET images of brain were acquired with a microPET Focus 220 scanner (Siemens Medical Solutions; Knoxville, TN) for 90 min with scan durations ranging from 30 s to 5 min. The position of the head was fixed using a stereotaxic frame. Electrocardiogram, body temperature, heart, and respiration rates were monitored throughout the experiment

2.8. Monkey plasma radiometabolite analysis

During monkey PET imaging in session 1 and 2, arterial blood samples were drawn for analysis of radiometabolites in plasma and for determination of a radiometabolite-corrected arterial input function. Samples (0.5–1 mL each) were drawn at 15 s intervals until 120 s, followed by 0.5- to 4-mL samples at 3, 5, 10, 30, 60, 90, and 120 min. The concentration of parent radioligand was measured with HPLC on an X-terra column (10 μm , 7.8×300 mm; Waters Corp.) eluted at 5.0 mL min^{-1} with methanol-water-triethylamine (85:15:0.1, by vol.), after separating plasma from whole blood and processing, as previously described [21].

2.9. Compartmental modeling of PET data

Images were reconstructed using Fourier rebinning plus two-dimensional filtered back-projection. PET images were co-registered to a standardized monkey MRI template using SPMS (Wellcome Trust Centre, London, UK). A set of 34 predefined brain regions of

interest from the template were then applied to the co-registered PET image to obtain regional decay-corrected time-activity curves. All PET images were corrected for attenuation and scatter. Uptake of radioactivity in each region of interest was expressed as SUV. Total distribution volumes (V_T) were estimated for different regions by two tissue compartmental modeling with PET brain time-activity curves and the measured radiometabolite-corrected arterial input function. The PET data analysis was performed using PMOD (PMOD Technologies Ltd.; Zurich, Switzerland).

2.10. Plasma free fraction determination

The plasma free fraction (f_p) of [^{11}C]4 was measured with ultrafiltration through membrane filters (Centrifree; Millipore), as previously described [22]. Briefly, formulated [^{11}C]4 (170 kBq at the time of counting in ~23 μL) was added to plasma (650 μL). The mixture was incubated at room temperature for 10 min and then processed as described previously. Radioactive ultra-filtration components were allowed time to decay within the optimal range of the γ -counter before being counted. Quantification of the ultra-filtrates was done gravimetrically.

2.11. Monkey PET imaging using [^{11}C]5

The PET study in nonhuman primate at Karolinska Institutet was approved by the Animal Ethics Committee of the Swedish Animal Welfare Agency (Dnr N185/14) and was performed according to the relevant guidelines of the Karolinska Institutet ("Guidelines for Planning, Conducting, and Documenting Experimental Research" (Dnr 4820/06–600) and "Guide for the Care and Use of Laboratory Animals".

A female cynomolgus monkey (5.5 kg) was supplied by Astrid Fagraeus Laboratory of the Swedish Institute for Infectious Disease Control (Solna, Sweden). Anesthesia was initiated via intramuscular injection of ketamine hydrochloride (ca 10 mg kg^{-1}) and maintained after endotracheal intubation by administration of a mixture of sevoflurane, oxygen and medical air. The monkey was observed continuously during the days of PET measurements. Body temperature was maintained by Bair Hugger-Medel 505 (Arizant Healthcare Inc., MN) and monitored with an esophageal thermometer. Heart and respiration rates were continuously monitored by using PC-VetGard™ system. The monkey head was fixed in position throughout PET scanning, as described previously [23]. For baseline PET scan [^{11}C]5 (146 MBq; 324 GBq mol^{-1}) was injected intravenously during 5 s with simultaneous start of PET data acquisition on the Siemens HRRT scanner. Radio-activity in brain was measured continuously for 123 min according to a preprogrammed series of 34 frames [24]. For the measurement of metabolites and plasma free fraction (f_p) manual blood samples (2 mL) were obtained at 4, 10, and then every 10 min. The f_p was determined by HPLC with radio-detection as previously described [25].

2.12. Rat PET imaging

In one experiment, three rats (Sprague Dawley; 213.3 ± 6.4 g) that had been anesthetized with ketamine (10 mg kg^{-1}) and then maintained in anesthesia with 1.5% isoflurane were used for PET scanning sessions after bolus intravenous administration of [^{11}C]4 (45 ± 1 MBq; 76 GBq μmol^{-1}). One rat was studied at baseline. One rat was studied under a P-gp-

preblocked condition, achieved by administering tariquidar (16 mg kg^{-1} , i.v.) at 25 min before [^{11}C]4 injection. In another rat, tariquidar (16 mg kg^{-1}) was administered 20 min before [^{11}C]4 injection and AR-A000002 (2 mg kg^{-1}) at 15 min after [^{11}C]4 injection.

In a separate experiment, three rats (Sprague Dawley; $346.0 \pm 15.5 \text{ g}$) were anesthetized with ketamine (10 mg kg^{-1}) and maintained in anesthesia with 1.5% isoflurane. They were used for PET scanning sessions with non-radioactive **4**, AR-A000002 and fenfluramine as treatments. Thus, after treatment of all rats with tariquidar (8 mg kg^{-1} , i.v.), one rat was injected intravenously with **4** (2 mg kg^{-1} , i.v.), another one with AR-A000002 (2 mg kg^{-1} , i.v.); and the third with fenfluramine (2 mg kg^{-1} , i.v.), all 15 min after injection of a bolus of [^{11}C]4 ($39.3 \pm 0.6 \text{ MBq}$; $36 \text{ GBq } \mu\text{mol}^{-1}$).

In each experiment, PET images of brain were acquired with a microPET Focus 220 scanner (Siemens Medical Solutions; Knoxville, TN) for 90 min. The PET data analysis was performed using PMOD (PMOD Technologies Ltd.; Zurich, Switzerland).

2.13. Ex vivo measurement of radiometabolites of [^{11}C]4 in rat brain

Tariquidar (8 mg kg^{-1}) was injected intravenously through the penile vein into three anesthetized rats ($269 \pm 53 \text{ g}$) at $6.7 \pm 1.2 \text{ min}$ before likewise injection of [^{11}C]4. The rats were anesthetized with 1.5% isoflurane in oxygen and sacrificed via thoracotomy at 30 min after injection. Anticoagulated blood was drawn from the myocardium and the rats were decapitated to harvest forebrain and cerebellum. Each tissue was removed, weighed, and immediately subjected to radioanalysis. All tissue assays were corrected for physical decay with a half-life of 20.385 min. The whole blood samples were centrifuged to separate the plasma. The plasma samples ($\sim 450 \mu\text{L}$) were then deproteinated with acetonitrile ($720 \mu\text{L}$). Whole blood ($50 \mu\text{L}$ each) and plasma samples ($50 \mu\text{L}$ each) were quantified for radioactivity as previously described [21] in an automatic - γ -counter (model 1480 Wizard; Perkin-Elmer) with an electronic window set at 360–1800 keV (counting efficiency = 51.84%). The radioactivity of brain tissue was also counted in the - γ -counter before homogenization in acetonitrile (3.0 mL) with a hand-held tissue Tearor (model 985–370; BioSpec Products Inc.) and after a second homogenization in the presence of added water (1.0 mL). These homogenates were counted in the - γ -counter to calculate the percentage recovery of radioactivity into the acetonitrile extracts. The homogenates were then centrifuged at $10,000g$ for 1 min. The clear supernatant was injected onto the HPLC column through nylon filters. Radioactivity in the resulting precipitate was used to calculate the percent recovery of activity in the acetonitrile supernatant.

Radio-HPLC analysis was performed on X-terra® C₁₈ ($10 \mu\text{m}$, $7.8 \text{ mm} \times 300 \text{ mm}$, Waters Corp., Milford, MA), eluted with methanol: water:Et₃N ($80:20:0.1$; by vol.) at 4.0 mL min^{-1} . Eluates were monitored with an in-line flow-through NaI(π) scintillation detector (Bioscan). Data were stored and analyzed on a PC using the software “Bio-ChromeLite”. Data in each radiochromatogram were decay-corrected to the respective HPLC injection time.

2.14. Ex vivo measurement of radiometabolites of [^{11}C]4 in P-gp/BCRP knock-out mice brain

P-gp/BCRP knockout (KO) mice (Taconic Farm, Germantown, NY) were anesthetized with 1.5% isoflurane and oxygen and sacrificed via thoracotomy at 30 min after radioligand injection. Heparinized (anticoagulated) blood was drawn from the myocardium, and the forebrains and cerebella were subsequently harvested. The tissues were weighed and immediately subjected to radioanalysis. Whole blood samples were centrifuged to separate the plasma. Plasma samples (300–350 μL) were then deproteinated with acetonitrile (720 μL). Whole blood (50 μL each) and plasma samples (50 μL each) were quantified for radioactivity, as previously described above in an automatic γ -counter. The radioactivities of brain tissues were also counted in the γ -counter before homogenization in acetonitrile (1.0–1.5 mL) using a hand-held tissue Tearor (model 985–370; BioSpec Products Inc.) and again after homogenization in the presence of water (0.5 mL). Anticoagulated blood (~1 mL; STD-A), plasma (STD-B) and fresh brain (1.3 g) were harvested from a control rat (409 g) and a control mouse (48.2 g). The brains of the control animals were harvested and immediately weighed and homogenized with twice their weights of ice cold saline. Plasma was separated from whole blood by centrifugation. A radioligand dose was then added to these three tissue samples from each animal before incubation in a shaking water bath at 37 °C for 1 h (plasma, blood and brain homogenate; 1.11 MBq). Aliquots (50 μL) were removed for radio-analysis and processed as detailed above.

2.15. Quantum chemical calculations

The geometry and energetics of compounds **1–5** in the presence of a single water molecule were obtained with density functional theory at the level of B3LYP/6–31G*. These quantum chemical calculations were all carried out in the reaction field of water with the polarizable continuum model as implemented in Gaussian 09 software [26].

3. Results

3.1. Chemistry

The structure of **4** lends itself to be labeled with carbon-11 in at least two positions by different methods, namely ^{11}C -carbonylation and ^{11}C -*N*-mesylation [27,28]. Both options were considered, and ^{11}C -carbonylation was selected mostly because of ready access to [^{11}C] carbon monoxide as labeling agent [29]. Two precursors were synthesized for use in a reaction of [^{11}C] carbon monoxide that couples a 4-iodophenyl methanesulfonate (**14**) with a requisite homochiral amine [(*R*)-**13**]. The iodide, rather than the bromide, was selected as a coupling partner to minimize the risk of Pd insertion into the aryl-sulfonate bond. **14** was prepared simply by mesylation of 4-iodophenol with methanesulfonyl chloride. (*R*)-**13** required a multistep synthesis with careful consideration on how to separate and analyze diastereomers (Scheme 1).

Starting from commercially available 8-methoxytetralone (**7**), reductive amination with benzylamine, using NaCNBH_3 as reducing agent, gave racemic **8** in 85% yield. A homochiral protecting group was then incorporated through treatment of the amine with (1*R*)-menthylchloroformate to afford a diastereomeric mixture (**9**) in 92% aggregate yield.

Only limited separation of the diastereomers could be achieved on silica gel. Therefore, the diastereomeric mixture was used in the next step. Demethylation of **9** with BBr₃ in dichloromethane (DCM) gave the desired pair of diastereomeric phenols (**10**) in 83% yield. These phenols were then treated with triflic anhydride and pyridine in DCM to give the corresponding pair of diastereomeric aryl triflates (**11**) in 68% yield. A Buchwald-Hartwig amination reaction between **11** and 1-methylpiperazine gave a mixture of diastereomers (**12**) from which (*R*)-**12** and (*S*)-**12** were separated by silica gel flash chromatography. The diastereomers were deprotected separately in two steps to give (*R*)-**13** and (*S*)-**13**. First, the (1*R*)-menthyl group was removed by heating the carbamate with HBr in formic acid and acetic acid in a sealed vial. The benzyl group was then removed with H₂ and 10% Pd on carbon. The desired homochiral amines [(*R*)-**13** and (*S*)-**13**] were each obtained in 90% yield. The specific optical rotations ($[\alpha]_D^{22}$) of the amines were compared with that of an authentic reference sample obtained from AstraZeneca. The analysis showed specific rotations ($[\alpha]_D^{22}$); for enantiomer (*R*)-**13** and the reference sample of +3.5 and +3.7 respectively, whereas enantiomer (*S*)-**13** had a specific rotation ($[\alpha]_D^{22}$); of -2.8.

3.2. Radiochemistry

3.2.1. Radiosynthesis of [¹¹C]4—[¹¹C]Carbon monoxide was produced from cyclotron-produced [¹¹C] carbon dioxide by passage over heated molybdenum [27]. 4-Iodophenyl methanesulfonate (**14**) was sonicated with Pd₂(dba)₃ and Xantphos in THF and injected under pressure into an autoclave along with the [¹¹C]carbon monoxide (Scheme 2). The mixture was heated for 5 min before being released into a vial containing the chiral amine (*R*)-**13** and tributylamine (TBA). The vial was heated under helium flow to evaporate residual solvent and generate the desired amide. This two-pot procedure gave fewer unidentified radioactive side-products than the one-pot procedure [30]. [¹¹C]4 was produced starting from [¹¹C] CO₂ in yields of $6.4 \pm 1.6\%$ (1240 ± 347 MBq; $n = 7$) within 50 min. [¹¹C]4 was then separated with reversed phase HPLC and formulated in saline (with 10% v/v EtOH) for intravenous injection. HPLC showed that the radioligand was radiochemically pure and free of any appreciable chemical impurities (see Supporting Information for radiochromatogram). Molar activities were 83 ± 51 GBq μmol^{-1} ($n = 7$) at end of synthesis.

3.2.2. Radiosynthesis of [¹¹C]5—Several experiments were performed to arrive at these conditions to produce this radioligand for single PET measurement in NHP. Cyclotron produced [¹¹C]methane was converted into [¹¹C]methyl iodide by high temperature iodination in a gas-phase recirculation system and subsequently transformed into [¹¹C]methyl triflate. [¹¹C]5 was obtained by trapping [¹¹C]methyl triflate in a solution of **6** in acetone (Scheme 3), isolated with reversed phase preparative HPLC (see Supporting Information for radiochromatogram), and formulated in saline in a sterile vial. The radioligand represented 70% of the radioactivity in the HPLC injectate. Formulated [¹¹C]5 (1025 MBq) was obtained in 98% radiochemical purity and with a molar activity of 654 GBq μmol^{-1} ($n = 1$) after 30 min total synthesis time after end of radionuclide production.

3.3. PET experiments with [^{11}C]4 in monkey

PET images (Fig. 2A/B) and time-activity curves (Fig. 3A) obtained after administration of [^{11}C]4 showed very low radioactivity uptake in monkey brain (<0.2 SUV) before and after blockade of 5-HT_{1B} receptors with AR-A000002 (AZD3783, 2 mg kg⁻¹, i.v.). AR-A000002, at an intravenously administered dose of 2 mg kg⁻¹ has previously been shown to block monkey brain 5-HT_{1B} receptors in PET experiments with other radioligands [31]. Tariquidar (TQD) is a P-glycoprotein (P-gp) inhibitor that may be administered intravenously to inhibit P-gp in vivo [32]. Tariquidar (8 mg kg⁻¹) increased brain radioactivity uptake to about 0.5 SUV. No blocking effect was observed after combining tariquidar and AR-A000002 administration, indicating absence of any specific 5-HT_{1B} receptor binding.

Very high radioactivity uptake in pituitary gland (> 6 SUV) was observed at baseline and under all other conditions. Administration of tariquidar or tariquidar plus AR-A000002 did not alter [^{11}C]4 uptake in pituitary gland (Fig. 2C/D) but uptake was markedly increased under 5-HT_{1B} receptor blockade (Fig. 3B).

3.4. Analysis of plasma in monkey PET experiments with [^{11}C]4

Following injection of [^{11}C]4 into monkey at baseline, radioactivity quickly decreased in plasma. Analysis of plasma showed five radiometabolites, [^{11}C]A-[^{11}C]E (see Fig. S5). The ability of these radiometabolites to enter brain is unknown. [^{11}C]E, which was the most lipophilic radiometabolite and the most likely able to enter brain, reached only 2.6% of radioactivity in plasma by 90 min after [^{11}C]4 injection. The time-courses of unchanged [^{11}C]4, in terms of contribution to percentage radioactivity in plasma and to unchanged [^{11}C]4 concentration (SUV), were similar at baseline and under 5-HT_{1B} receptor blocked conditions (Fig. 4A). The curves in Fig. 4B constitute the radiometabolite-corrected arterial input functions for use in compartmental modeling.

3.5. Compartmental modeling of monkey PET data from [^{11}C]4

One-tissue (1TC) and two-tissue (2TC) compartmental models were used with PET data and arterial input functions to derive whole brain total volumes of distribution (V_T s) under four types of monkey condition (Fig. 5). By either model, V_T values at baseline were very low and did not increase appreciably under 5-HT_{1B} receptor blocking condition. Pretreatment with tariquidar increased V_T by either model, which indicates [^{11}C]4 is possibly a substrate for the efflux transporter P-gp. However, the radiometabolites are also possibly P-gp substrates. Therefore, the increased radioactivity in brain could have significant contributions from radiometabolites.

Blockade of 5-HT_{1B} receptors under P-gp pre blocked conditions did not reduce V_T in either model (Fig. 5), showing again absence of any clear receptor-specific signal. Both models provided essentially the same V_T data.

3.6. PET experiments with [^{11}C]4 in rat

After injection of [^{11}C]4 into Sprague Dawley rats, PET showed low uptake of radioactivity into brain. Brain uptake was higher in rats pretreated with tariquidar, but this uptake was not

displaced by administration of 5-HT_{1B} receptors ligands. No 5-HT_{1B} receptor specific binding could be detected in rats treated with tariquidar and AR-A000002 (Fig. 6A). These results parallel those in monkey. Furthermore, treatment of rats with **4** or AR-A000002 at 15 min after radioligand injection had no effect on brain time-activity curves (Fig. 6B). Administration of fenfluramine at 15 min after radioligand injection to release serotonin into the synaptic cleft showed no clearance of activity from brain. The tariquidar dose was initially 16 mg kg⁻¹ but was reduced to 8 mg kg⁻¹ in the second set of PET imaging experiment due to safety concerns for the rats.

3.7. Ex vivo experiments in rats and mice with [¹¹C]**4**

In tariquidar-treated rats, plasma radioactivity was $43.7 \pm 3.2\%$ ($n = 3$) unchanged parent radioligand at 30 min after injection. Radioactivity in ex vivo forebrain and cerebellum of these rats was respectively $99.7 \pm 0.4\%$ ($n = 3$) and $98.8 \pm 0.5\%$ ($n = 3$) unchanged parent radioligand at 30 min after injection. The forebrain uptake was 0.8 ± 0.1 SUV ($n = 3$) and the cerebellum uptake was 1.0 ± 0.1 SUV ($n = 3$).

In P-gp/BCRP knockout (KO) mice, the ex vivo plasma composition was $43.2 \pm 0.8\%$ ($n = 3$) parent radioligand at 30 min after injection. At this time, radioactivity in ex vivo forebrain and cerebellum of KO mice was $97.0 \pm 1.0\%$ ($n = 3$) and $99.0 \pm 0.4\%$ ($n = 3$) parent radioligand, respectively. There were no regional differences in the distribution of [¹¹C]**4** in mouse brain. The forebrain uptake was 0.6 ± 0.2 SUV ($n = 3$) and the cerebellum uptake was 0.7 ± 0.1 SUV ($n = 3$).

3.8. Apparent pK_a, lipophilicity, and plasma free fraction of [¹¹C]**4**

To probe for the underlying reasons behind the poor brain entry of [¹¹C]**4**, apparent pK_a, lipophilicity, and plasma free fraction were measured for [¹¹C]**4**. [¹¹C]**4** was $99.0\% \pm 0.1\%$ ($n = 2$) stable in sodium phosphate buffer (pH 7.4) for at least 2.5 h. There was no adsorption to the walls of glass test tubes from buffer ($<0.8 \pm 0.6\%$; $n = 2$) or human plasma ($<1.4\%$).

3.8.1. Apparent pK_a—The apparent pK_a of [¹¹C]**4** was 8.00 ± 0.03 ($n = 3$), based on a method distributing [¹¹C]**4** between cyclohexane and buffer [33]. This shows that, according to the Henderson-Hasselbalch equation, the radioligand would be protonated to a large extent (79.9%) at physiological pH(7.4).

3.8.2. Lipophilicity (logD_{7.4})—The measured logD_{7.4} of [¹¹C]**4** at room temperature in 1-octanol was 2.01 ± 0.01 ($n = 6$).

3.8.3. Plasma free fraction (f_p)—The plasma free fraction (f_p) of [¹¹C]**4** in human plasma was $18.0 \pm 0.8\%$ ($n = 3$) and reflects the moderate lipophilicity of the radioligand [34].

3.9. PET experiments with [¹¹C]**5** in monkey

[¹¹C]**5** was studied at baseline in one female cynomolgus monkey. Upon administration of radioligand, brain uptake reached only 0.6 SUV and slowly decreased to 0.25 SUV over the

123-min duration of scanning. No differences in brain region uptakes were detected (Fig. 7). High radioactivity uptake in the pituitary was also observed.

3.10. Analysis of plasma in monkey PET experiments with [^{11}C]5

Analysis of plasma following injection of [^{11}C]5 showed one major radiometabolite and two minor radiometabolites (see Fig. S6). Parent compound decreased to 30% after 60 min. All radiometabolites were less lipophilic than parent compound and it is unlikely they would enter the brain to any greater extent. The plasma free fraction was greater than for [^{11}C]4 and was measured to be $50.30 \pm 0.03\%$ ($n = 6$).

3.11. Structure, P-gp substrate behavior and ability to cross the BBB

The measured apparent pK_a of **4** (8.00) indicates the *N*-methyl-piperazinyl ring will be mostly (79.9%) protonated at physiological pH . This should be true for an *N*-methyl-piperazinyl ring in similar structures, such as [^{11}C]1- ^{11}C]5. After intravenous injection, the non-protonated forms of [^{11}C]1- ^{11}C]5 should be well represented in plasma (20%) and have ability to diffuse across the BBB (blood-brain barrier), in the absence of efflux transporter action.

Generally, charged species have very low ability to cross the BBB, except when the formal charge is spread across the molecule. We considered that ligands **1–3** might form an internal hydrogen bond (H-bond) with the amide nitrogen, potentially to delocalize charge, and so confer ability to cross the BBB. To investigate such a possibility, we performed quantum chemical calculations at the level of B3LYP/6–31G* to obtain the structures and energetics for compounds **1–5** (Table 1 and Fig. S7). To single out the effect of the potential hydrogen bond between the amide and piperazine nitrogen, these calculations were all carried out with the protonated *N*-methyl-piperazine plus a water molecule (Fig. 8) in the reaction field of water.

Conformer **1b** was obtained by varying the dihedral angle (φ_1) centered on the C—N bond of **1a** with the *N*-methyl proton facing the bicyclic ring (Fig. 8). Conformer **1c** was generated by further varying the dihedral angle (φ_2) centered on the C—C bond of **1b** making its amide oxygen form an H-bond with the water molecule (Fig. 8). The energy minimized structure of **1b** is less stable than **1a** by ΔG of 2.3 kcal mol $^{-1}$. Of the three conformers, **1c** is most stable due to the water molecule forming H-bonds with both the *N*-methyl proton and the amide oxygen. The conformer **1b** lacking such a water-bridge is less stable than **1a** that H-bonds with the water molecule in an open space. It is noted that in the absence of a water molecule, **1c** is the least energetically favorable due to the steric repulsion caused by the amide oxygen to the neighboring ring oxygen atom.

4. Discussion

Absence of receptor-specific signal might be due to one or more of several reasons. For example: (1) radio ligand is not entering brain even under P-gp blocked condition; (2) the radioligand will only bind to 5-HT $_1B$ receptors that are in the G-protein coupled state, which may exist in relatively much lower density than the total population of 5-HT $_1B$ receptors

seen by antagonists; or (3) the off-rate of the radioligand from the G-protein coupled receptor is too fast.

The apparent pK_a is attributable to the piperazinyl ring, most likely at the *N*-methyl position [35]. The *N*-methyl-piperazinyl group features in many effective PET radioligands, such as the 5-HT_{1B} receptor ligands shown in Fig. 1. Also many effective 5-HT_{1A} receptor PET radioligands have an aryl piperazine sub-structures, notably [¹¹C]WAY-100635 ([¹¹C]WAY) [36] and [¹¹C]DWAY [37]. Therefore, the pK_a of **4** does not appear to be an impediment to brain entry by passive diffusion [38].

Because **4** has formal properties consistent with ability to penetrate the BBB [17], and because **4** is structurally related to other BBB-penetrant radioligands [39], we suspected that [¹¹C]**4** is prevented from entering brain by efflux transporters [40]. The lipophilities, pK_a and $\log P$ values of [¹¹C]**4** and [¹¹C]**5** appeared all to be conducive to conferring good ability to penetrate the BBB by passive diffusion. In the case of [¹¹C]**4** at least one efflux transporter, namely P-gp, was found to limit BBB penetration. This may also be true for [¹¹C]**5**, although this was not tested. The efflux transporter liability of [¹¹C]**4** is somewhat surprising given that its structure resembles radioligands that do enter brain quite well, such as [¹¹C]**1–3**. A comparison of the structures of [¹¹C]**4** and [¹¹C]**5** with previous BBB-penetrant 5-HT_{1B} receptor radioligands [¹¹C]**1** and [¹¹C]**3** reveals notable differences, such as the lack of planarity and heteroatoms in the bicyclic ring systems of [¹¹C]**4** and [¹¹C]**5**, and the reversed amide bond (Fig. 1). Nonetheless, these structural features are also present in AR-A000002, which because it can be used as a brain 5-HT_{1B} blocking agent, clearly has some ability to enter brain after intravenous administration of a pharmacological dose. In the study by Andersson et al. [39], a radiolabeled 5-HT_{1B} receptor ligand (**15**, Fig. 9), having the same bicyclic ring system and amide direction as P [¹¹C]**3**, failed to enter monkey brain to any measurable extent after intravenous injection of a microdose.

The comparison of both energetics and structures of **1–5** suggests that the protonated *N*-methyl-piperazinyl group makes compounds **1–3** more amenable to BBB penetration by internal H-bonding mediated by a water molecule. This internal H-bonding may well confer thermochemical stability to their closed conformation while attenuating the charge on the protonated *N*-methyl-piperazinyl. The BBB-penetrant compounds **1–3** can form energetically favorable water-bridged conformations in terms of both enthalpy (ΔH) and Gibbs free energy (ΔG) whereas for the low-BBB-penetrating compounds **4–5** the open conformations are more stable in terms of ΔG . This suggests that under physiological conditions the piperazinyl nitrogen is fully charged and would hinder BBB penetration by passive diffusion. Our quantum chemical calculations further indicate that the water-bridged conformation also attenuates the charge on the *N*-methyl as evidenced by its smaller dipole moment than the open conformation as listed in Table 1. Considering the most energetically favorable conformation (lowest ΔG) of all compounds (**1c**, **2b**, **3c**, **4a** and **5a**), it is clear that **4a** and **5a** have the highest dipole moments and would be least likely to cross passively through the BBB.

Another possible reason why the full agonists [¹¹C]**4** and [¹¹C]**5** do not have useful uptake into monkey brain after intravenous injection is simply that there is no appreciable binding

to the brain 5-HT_{1B} receptor in the G-protein-coupled state. Any binding may be too weak or brief to keep the radioligand in the brain. This suggestion accords with the data presented herein. Further study using a full agonist 5-HT_{1B} receptor ligand that has good uptake into brain by passive diffusion is needed to reinforce this suggestion. Attempts to develop full agonist PET radioligands for imaging the highly homologous 5-HT_{1A} receptor have also met with limited success [41,42].

5. Conclusions

The focus of this paper was on evaluating full agonist PET radioligands for their potential to image the brain 5-HT_{1B} receptor in the G-protein coupled state. Although the full 5-HT_{1B} receptor agonist radioligands, [¹¹C]**4** and [¹¹C]**5**, were successfully prepared, they showed limited ability to enter brain after intravenous injection and provided no useful signal for imaging with PET. Our investigation of several different reasons for the poor brain uptake and lack of specific binding, gave no one clear explanation. Nonetheless, our examination of ligand conformational energies with regards to internal H-bonds and their ability to attenuate the formal charge of the molecule and affect passive diffusion through the BBB shows some interesting results. Such factors are worth further exploring with a larger cohort of compounds. Further studies would require a BBB-penetrant full agonist PET radioligands to refute or confirm that a full agonist is viable as a 5-HT_{1B} full agonist PET radioligand.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- [1]. Moret C, Briley M. The possible role of 5-HT_{1B/D} receptors in psychiatric disorders and their potential as a target for therapy. *Eur J Pharmacol* 2000;404: 1–12.
- [2]. Cunha L, Szigeti K, Mathe D, Metello LF. The role of molecular imaging in modern drug development *Drug Discov Today* 2014; 19:936–48.
- [3]. Paterson LM, Komum BR, Nutt DJ, Pike VW, Knudsen GM. 5-HT radioligands for human brain imaging with PET and SPECT. *Med Res Rev* 2013;33:54–111. [PubMed: 21674551]
- [4]. Vamas K, Hall H, Bonaventure P, Sedvall G. Autoradiographic mapping of 5-HT_{1B} and 5-HT_{1D} receptors in the post mortem human brain using H-3 GR 125743. *Brain Res* 2001;915:47–57. [PubMed: 11578619]
- [5]. Ruf BM, Bhagwagar Z. The 5-HT_{1B} receptor: a novel target for the pathophysiology of depression. *Curr Drug Targets* 2009;10:1118–38. [PubMed: 19702551]
- [6]. Hannon J, Hoyer D. Molecular biology of 5-HT receptors. *Behav Brain Res* 2008; 195: 198–213. [PubMed: 18571247]

- [7]. Hoyer D, Clarke, Fozard JR, Hartig PR, Martin GR, Mylecharane EJ, et al. International union of pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). *Pharmacol Rev* 1994;46:157–203.
- [8]. Clawges HM, Depree KM, Parker EM, Graber SG. Human 5-HT₁ receptor subtypes exhibit distinct G protein coupling behaviors in membranes from Sf9 cells. *Biochemistry* 1997;36:12930–8. [PubMed: 9335552]
- [9]. Brys R, jossion K, Castelli MP, Jurzak M, Ujnen P, Gommeren W, et al. Reconstitution of the human 5-HT_{1D} receptor-G-protein coupling: evidence for constitutive activity and multiple receptor conformations. *Mol Pharmacol* 2000;57:1132–41.
- [10]. Kenakin T. ligand-selective receptor conformations revisited: the promise and the problem. *Trends Pharmacol Sci* 2003;24:346–54.
- [11]. Kobilka BK G protein coupled receptor structure and activation. *BBA-Biomembranes* 2007;1768:794–807. [PubMed: 17188232]
- [12]. Bhagwagar Z, Ruf B, Gallezot JD, Hu J, Pittman B, Krystal J, et al. Test-retest reliability of the novel PET radioligand 11C–P943 as a measure of 5-HT_{1B} receptor binding. *Biol Psychiatry* 2008;63:184S.
- [13]. Pierson ME, Andersson J, Nyberg S, McCarthy DJ, Finnema SJ, Varnas K, et al. (11)CAZ1 0419369: a selective 5-HT_{1B} receptor radio ligand suitable for positron emission tomography (PET). Characterization in the primate brain. *Neuroimage* 2008;41:1075–85. [PubMed: 18434202]
- [14]. Lindberg A, Nag S, Schou M, Takano A, Matsumoto J, Amini N, et al. [(11)C] AZ1 0419096-a full antagonist PET radioligand for imaging brain 5-HT_{1B} receptors. *Nucl Med Biol* 2017;54:34–40.
- [15]. Paterson LM, Tyacke RJ, Nutt DJ, Knudsen GM. Measuring endogenous 5-HT release by emission tomography: promises and pitfalls. *J Cereb Blood Flow Metab* 2010;30: 1682–706. [PubMed: 20664611]
- [16]. Finnema SJ, Varrone A, Hwang TJ, Gulyas B, Pierson ME, Halldin C, et al. Fenfluramine-induced serotonin release decreases C-11 AZ10419369 binding to 5-HT_{1B}-receptors in the primate brain. *Synapse* 2010;64:573–7. [PubMed: 20222157]
- [17]. Pike VW. Considerations in the development of reversibly binding PET radioligands for brain imaging. *Curr Med Chem* 2016;23:1818–69.
- [18]. Andersson J, Truong P, Halldin C. In-target produced C-11 methane: increased specific radioactivity. *Appl Radiat Isot* 2009;67:106–10. [PubMed: 19013077]
- [19]. Larsen P, Ulin J, Dahlstrom K, Jensen M. Synthesis of C-11 iodomethane by iodination of C-11 methane. *Appl Radiat Isot* 1997;48:153–7.
- [20]. Clark J, Baldwin R, Bayne K, Brown M, Gebhart G, Gonder J. Guide for the care and use of laboratory animals.. 8th ed. National Academies Press; 2008.
- [21]. Zoghbi SS, Shetty HU, Ichise M, Fujita M, Imaizumi M, Liow JS, et al. PET imaging of the dopamine transporter with F-18-FECNT: a polar radiometabolite confounds brain radio ligand measurements. *J Nucl Med* 2006;47:520–7.
- [22]. Gandelman MS, Baldwin RM, Zoghbi SS, Zea-Ponce Y, Innis RB. Evaluation of ultrafiltration for the free-fraction determination of single-photon emission computed-tomography (SPECT) radiotracers -β-CIT, IBF, and iomazenil. *J Pharm Sci* 1994;83: 1014–9. [PubMed: 7965658]
- [23]. Karlsson P, Farde L, Halldin C, Swahn CG, Sedvall G, Foged C, et al. PET examination of C-11 NNC-687 and C-11 NNC-756 as new radioligands for the D-1-dopamine receptor. *Psychopharmacology* 1993;113:149–56.
- [24]. Varrone A, Sjöholm N, Eriksson L, Gulyas B, Halldin C, Farde L. Advancement in PET quantification using 3D-OP-OSEM point spread function reconstruction with the HRRT. *Eur J Nucl Med Mol Imaging* 2009;36:1639–50.
- [25]. Moein MM, Nakao R, Amini N, Abdel-Rehim M, Schou M, Halldin C. Sample preparation techniques for radiometabolite analysis of positron emission tomography radioligands; trends, progress, limitations and future prospects. *Trac-Trends in Anal Chem* 2019;110:1–7.
- [26]. Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR. et al. Gaussian 09 revision. A. 2nd ed. Wallingford CT: Gaussian Inc.; 2009.

- [27]. Rahman O. C-11 carbon monoxide in labeling chemistry and positron emission tomography tracer development: scope and limitations. *J Label Compd Radiopharm* 2015;58:86–98.
- [28]. McCarron JA, Pike VW. Synthesis of no-carrier-added C-11 methanesulfonyl chloride as a new labeling agent for PET radiopharmaceutical development *J Label Compd Radiopharm* 2003;46:1127–40.
- [29]. Lu SY, Hong J, Itoh T, Fujita M, Inoue O, Innis RB, et al. Carbonyl-C-11 benzyl acetate: automated radiosynthesis via Pd-mediated C-11 carbon monoxide chemistry and PET measurement of brain uptake in monkey. *J Label Compd Radiopharm* 2010;53: 548–51.
- [30]. Hong J, Lu SY, Xu R, Liow JS, Wock AE, Jenko KJ, et al. carbonyl-C-11 4-Fluoro-N-methyl-N-(4-(6-(methylamino)pyrimidin-4-yl)thiazol-2-yl)benzamide (C-11 FIMX) is an effective radioligand for PET imaging of metabotropic glutamate receptor 1 (mGluR1) in monkey brain. *Nucl Med Biol* 2015;42:967–74. [PubMed: 26320813]
- [31]. Varnas K, Nyberg S, Karlsson P, Pierson ME, Kagedal M, Cselenyi Z, et al. Dose-dependent binding of AZD3783 to brain 5-HT_{1B} receptors in non-human primates and human subjects: a positron emission tomography study with C-11 AZ10419369. *Psychopharmacology* 2011;213:533–45.
- [32]. Roe M, Folkes A, Ashworth P, Brumwell J, Chima L, Hunjan S, et al. Reversal of P-glycoprotein mediated multidrug resistance by novel anthranilamide derivatives. *Bioorg Med Chem Lett* 1999;9:595–600.
- [33]. Lacivita E, Leopoldo M, De Giorgio P, Berardi F, Perrone R. Determination of 1-aryl-4-propylpiperazine pK(a) values: the substituent on aryl modulates basicity. *Bioorg Med Chem* 2009;17:1339–44.
- [34]. Zoghbi SS, Anderson KB, Jenko KJ, Luckenbaugh DA, Innis RB, Pike VW. On quantitative relationships between drug-like compound lipophilicity and plasma free fraction in monkey and human. *J Pharm Sci* 2012;101:1028–39. [PubMed: 22170327]
- [35]. Khalili F, Henni A, East AIL pK(a) values of some piperazines at (298, 303, 313, and 323) K. *J Chem Eng Data* 2009;54:2914–7.
- [36]. Hume S, Hirani E, Opacka-Juffry J, Myers R, Townsend C, Pike V, et al. Effect of 5-HT on binding of C-11 WAY 100635 to 5-HT_{1A} receptors in rat brain, assessed using in vivo microdialysis and PET after fenfluramine. *Synapse* 2001;41:150–9. [PubMed: 11400181]
- [37]. Andree B, Hall din C, Pike VW, Gunn RN, Olsson H, Farde L, The PET radio ligand carbonyl-C-11 desmethyl-WAY-100635 binds to 5-HT_{1A} receptors and provides a higher radioactive signal than carbonyl-C-11 WAY-100635 in the human brain. *J Nud Med* 2002;43:292–303.
- [38]. Pike VW. PET radiotracers: crossing the blood-brain barrier and surviving metabolism. *Trends Pharmacol Sci* 2009;30:431–40.
- [39]. Andersson JD, Pierson ME, Finnema SJ, Gulyas B, Heys R, Elmore CS, et al. Development of a PET radioligand for the central 5-HT_{1B} receptor: radiosynthesis and characterization in cynomolgus monkeys of eight radiolabeled compounds. *Nud Med Biol* 2011;38:261–72.
- [40]. Dj Begley. ABC transporters and the blood-brain barrier. *Curr Pharm Des* 2004;10: 1295–312. [PubMed: 15134482]
- [41]. Lu S, Uow J-S, Zoghbi SS, Hong J, Innis RB, Pike VW. Evaluation of C-11 WAY-100635 and C-11 WAY-100635 in rat and monkey as agonist PET radioligands for brain 5-HT(1A) receptors. *Curr Radiopharm* 2010;3:9–18. [PubMed: 20657759]
- [42]. Vidal B, Fieus S, Colom M, Billard T, Bouillot C, Barret O, et al. F-18-F13640 preclinical evaluation in rodent, cat and primate as a 5-HT_{1A} receptor agonist for PET neuroimaging. *Brain Struct Funct* 2018;223:2973–88. [PubMed: 29730825]

Fig. 1.

Three PET radioligands successfully used for imaging human brain 5-HT_{1B} receptors ([¹¹C]**1–3**), and two candidate radioligands [¹¹C]**4–5**. K_i values, $clogD_{7.4}$ values and intrinsic activities are shown for comparison.

Fig. 2. Summed transaxial PET images (0–90 min) of monkey brain: (A) after intravenous administration of $[^{11}\text{C}]\mathbf{4}$ at baseline; (B) after blocking 5-HT_{1B} receptors with AR-A000002; (C) after blocking P-gp with tariquidar; and (D) after blocking 5-HT_{1B} receptors with AR-A000002 and P-gp with tariquidar.

Fig. 3.

Panel A: whole brain time-activity curves in monkey after intravenous administration of (¹¹C)4 (i) at baseline (■). (ii) after blocking 5-HT_{1B} receptors with AR-A000002 (2.0 mg kg⁻¹) (□). (iii) after blocking P-gp with tariquidar (8.0 mg kg⁻¹) (●), and (iv) after blocking 5-IIT_{1B} receptors with AR-A000002 (2.0 mg kg⁻¹) and P-gp with tariquidar (8.0 mg kg⁻¹) (○). Panel B: time-activity curves in monkey pituitary after intravenous administration of (¹¹C)4 (i) at baseline (▲) and (ii) after blocking 5-IIT_{1B} receptors with AR-A000002 (2.0 mg kg⁻¹) (▼).

Fig. 4. Measurement of $[^{11}\text{C}]4$ in monkey plasma at baseline and under 5-HT_{1B} receptor blocking conditions. Panel A: time-course of percentage of radioactivity represented by unchanged $[^{11}\text{C}]4$. Panel B: Time course of unchanged $[^{11}\text{C}]4$.

Fig. 5. Monkey whole brain V_T increases for [¹¹C]4 administered after tariquidar treatment Key: 1TC, 1-tissue compartmental model; 2TC, 2-tissue compartmental mode; BL, baseline condition; AR, after treatment with AR-A000002 (2.0 mg kg⁻¹); TQD, after treatment with tariquidar (8.0 mg kg⁻¹); TQD + AR, after treatment with tariquidar (8.0 mg kg⁻¹) and AR-A000002 (2.0 mg kg⁻¹).

Fig. 6.

Whole brain time-activity curves in rats intravenously administered with $[^{11}\text{C}]\mathbf{4}$. Panel A: at baseline (■), under P-gp block with tariquidar (16 mg kg^{-1}) (●) or under P-gp block with tariquidar (16 mg kg^{-1}) and AR-A000002 (2.0 mg kg^{-1}) (▲) given at 15 min after radioligand injection. Panel B: under P-gp block with tariquidar (8.0 mg kg^{-1}) and with $\mathbf{4}$ (2.0 mg kg^{-1}) (self, □), AR-A000002 (2.0 mg kg^{-1}) (○) or fenfluramine (2.0 mg kg^{-1}) (△) given at 15 min after radioligand injection.

Fig. 7. Summed transaxial PET images (0–123 min) of brain after intravenous administration of [^{11}C]**5** into monkey at baseline.

Fig. 8.
Geometry optimized structures of conformers of the complex of the protonated *N*-methyl-piperazine and a water molecule. ϕ_1 and ϕ_2 are the dihedral angles centered on the respective C—N bond of **1a** and C—C bond of **1b**. Atoms are colored as follows: white, hydrogen; green, carbon; blue, nitrogen; red, oxygen. Dashed lines indicate the H-bonding distances (<2.0 Å).

Fig. 9.
Examples of the small structural differences between BBB-penetrant and non-BBB-penetrant 5-HT_{1B} receptor ligands.

Scheme 1.

Synthesis of (*R*)-8-(4-methylpiperazin-1-yl)-1,2,3,4-tetrahydronaphthalen-2-amine [(*R*)-**13**]

Scheme 2.
Radiosynthesis of $[^{11}\text{C}]\text{4}$.

Scheme 3.
Radiosynthesis of $[^{11}\text{C}]\mathbf{5}$.

Table 1

Energetics and dipole moment of the conformers of compounds **1–5** calculated at the level of B3LYP/6–31G*.

Conformer	Enthalpy (H) ^a (kcal mol ^{−1})	Gibbs free energy [G] ^a (kcal mol ^{−1})	Dipole moment (Debye)
1a	0	0	30.9
1b	2.1	23	24.4
1c	−1.6	−1.2	18.0
2a	0	0	22.5
2b	−2.3	−0.9	15.5
3a	0	0	32.1
3b	2.9	3.4	25.1
3c	−2.0	−1.2	20.3
4a	0	0	24.1
4b	0.7	3.6	18.0
4c	−1.0	1.8	16.3
5a	0	0	30.9
5b	−0.2	1.8	14.4
5c	−1.8	0.2	12.6

^aRelative energetics of the respective conformers with respect to **1a**, **2a**, **3a**, **4a** and **5a** at 298.15 K. Geometry optimized structures of **2–5** are shown in Fig. S7.