Synthesis and evaluation of $^{18}$F-labeled 5-HT$_{2A}$ receptor agonists as PET ligands

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Introduction: The serotonin 2A receptor (5-HT$_{2A}$R) is the most abundant excitatory 5-HT receptor in the human brain and implicated in various brain disorders such as schizophrenia, depression, and Alzheimer’s disease. Positron emission tomography (PET) can be used to image specific proteins and processes in the human brain and several 5-HT$_{2A}$R PET antagonist radioligands are available. In contrast to an antagonist radioligand, an agonist radioligand should be able to image the population of functional receptors, i.e., those capable of inducing neuroreceptor signaling. Recently, we successfully developed and validated the first 5-HT$_{2A}$R agonist PET tracer, $[^{11}$C$]$Cimbi-36, for neuroimaging in humans and herein disclose some of our efforts to develop an $^{18}$F-labeled 5-HT$_{2A}$R agonist PET-ligand.

Methods and results: Three fluorine containing derivatives of Cimbi-36 were synthesized and found to be potent 5-HT$_{2A}$R agonists. $^{18}$F-labeling of the appropriate precursors was performed using $[^{18}$F$]$FETos, typically yielding 0.2–2.0 GBq and specific activities of 40–120 GBq/mmol. PET studies in Danish landrace pigs revealed that $[^{18}$F$]$Cimbi-36 displayed brain uptake in 5-HT$_{2A}$R rich regions. However, high uptake in bone was also observed. No blocking effect was detected during a competition experiment with a 5-HT$_{2A}$R selective antagonist. $[^{18}$F$]Cimbi-36$ showed very low brain uptake.

Conclusion: None of the investigated $^{18}$F-labeled Cimbi-36 derivatives $[^{18}$F$]Cimbi-36$, $[^{18}$F$]Cimbi-36$ and $[^{18}$F$]Cimbi-36$ show suitable tracer characteristics for in vivo PET neuroimaging of the 5-HT$_{2A}$R. Although for $[^{18}$F$]Cimbi-36$ there was reasonable brain uptake, we suggest that a large proportion radioactivity in the brain was due to radiometabolites, which would explain why it could not be displaced by a 5-HT$_{2A}$R antagonist.

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1. Introduction

The serotonergic neurotransmitter system plays a key role in several diseases and physiological processes such as depression, Alzheimer’s disease, schizophrenia, appetite, emotion, and the regulation of the sleep/wake cycle [1–3]. The serotonin 2A receptor (5-HT$_{2A}$R) is the primary target of psychedelic compounds such as LSD and psilocybin and 5-HT$_{2A}$R antagonism is a key feature of atypical antipsychotics.

Positron emission tomography (PET) is a quantitative and highly sensitive imaging tool for neuroimaging in humans. It plays a central role in disease management: diagnosis, staging, stratification, therapy planning and therapy monitoring. Additionally, functional brain imaging can determine receptor availability and occupancy, which can be used to make critical drug discovery and development decisions [4].

Currently, several antagonist 5-HT$_{2A}$R PET tracers are available, e.g., $[^{18}$F$]Alfotanerin, [^{11}$C$]MDL 100907 or (R)-[^{18}$F$]MH.MZ [5–8]. Radiolabeled agonists could have some advantages in regards to functional imaging for the following reason: according to the extended ternary complex model, agonists only bind to receptors in the high-affinity state, whereas antagonists label the whole population of receptors. Thus, agonists provide a good indication of the total population of available receptors, whereas radiolabeled agonists image the population of receptors, which are able to induce neuroreceptor signaling [8–11]. In addition, agonists should be more sensitive to endogenous neurotransmitter release and thus, be used to quantify release and/or depletion of neurotransmitters in vivo [8,10,12,13].

Recently, we evaluated a series of $^{11}$C-labeled 5-HT$_{2A}$R agonists as tracers [14]. $[^{11}$C$]Cimbi-36$, the most promising candidate, was further evaluated in non-human primates [15]. A fenfluramine challenge (inducing 5-HT release into the synaptic cleft) was able to reduce the specific binding of $[^{11}$C$]Cimbi-36$ in cortical non-human primate brain regions. $[^{11}$C$]Cimbi-36$ was subsequently evaluated in humans [16].
Pretreatment with the 5-HT\textsubscript{2A}R antagonist, ketanserin, significantly decreased \textsuperscript{11}C[Cimbi-36 binding in all cortical regions with no effects in cerebellum. These results confirm that \textsuperscript{11}C[Cimbi-36 binding is selective for 5-HT\textsubscript{2A}R and that cerebellum is an appropriate reference tissue for quantification of 5-HT\textsubscript{2A}Rs in the human brain.

Herein, we detail our efforts to develop an \textsuperscript{18}F-version of \textsuperscript{11}C[Cimbi-36 as fluorine-18 has several advantages from a clinical perspective. Sequentially substituting the three methoxy groups in Cimbi-36 with a \textsuperscript{18}F-fluoroethoxy moiety providing the three ligands \textsuperscript{18}F[1], \textsuperscript{18}F[2] and \textsuperscript{18}F[3] shown in Fig. 1. We have previously presented the in vivo evaluation of \textsuperscript{18}F[2] and \textsuperscript{18}F[3] \cite{17} and here we report the full details on the synthesis, radiosynthesis and evaluation of all three ligands. Very recently, the synthesis and in vitro evaluation of 3 and \textsuperscript{18}F[3] was reported by Prabhakaran et al. \cite{18}.

2. Materials and methods

2.1. Chemicals and reagents

Chemicals were purchased from Sigma-Aldrich. Unless otherwise stated, all chemicals were used as received.

2.2. Precursor synthesis

The synthesis of the precursors 10, 11 and 12 was recently described \cite{14,19,20}.

2.3. Reference compound synthesis

2.3.1. Synthesis of 4-(2-fluoroethoxy)-2-methoxybenzaldehyde (6). To a solution of 4-(2-fluoroethoxy)-2-methoxybenzaldehyde (6), 1.1 g, 5.7 mmol) was dissolved in MeNO\textsubscript{2} (19 mL) and added dropwise. The mixture was stirred at room temperature for 30 min and then sodium dithionite was added. The mixture was filtered and evaporated. Purification using column chromatography (5:5:90 MeOH:DMSO:EtOAc) to yield 0.380 g, 7% over 4 steps; \( R_f = 0.6 \) (EtOAc-n-Hep, 1:1).

2.3.1.2. Synthesis of 5-(2-fluoroethoxy)-2-methoxybenzaldehyde (5). To a solution of 5-(2-fluoroethoxy)-2-methoxybenzaldehyde (5) was added dropwise. The mixture was stirred at room temperature for 30 min and then sodium dithionite was added. The resulting mixture was stirred for 30 min, then evaporated, dissolved in EtOAc and washed with sat. NaHCO\textsubscript{3} until there was no remaining m-CBPA. The crude mixture was used without further purification. \( R_f = 0.65 \) (EtOAc-n-Hep, 1:1).

2.3.1.3. Synthesis of 5-(2-fluoroethoxy)-2-methoxybenzaldehyde (6). To a solution of 5-(2-fluoroethoxy)-2-methoxybenzaldehyde (6), 1.1 g, 5.7 mmol) was dissolved in DMF (20 mL) and added Et\textsubscript{3}N (7.4 mmol) and K\textsubscript{2}CO\textsubscript{3} (0.8 g, 5.7 mmol). The mixture was stirred at RT for 16 h, then water (100 mL) was added and the result extracted with EtOAc (3 × 30 mL), dried and evaporated. The crude mixture was used without further purification. \( R_f = 0.63 \) (EtOAc-n-Hep, 1:1).

2.3.1.4. Synthesis of (E)-4-(2-fluoroethoxy)-1-methoxy-2-(2-nitrovinyl)benzene (7). To a solution in THF (2 mL) of 

![Fig. 1. \textsuperscript{18}F-labeled analogues of Cimbi-36 investigated in this study.](image-url)
conversion (as indicated by TLC). The resulting mixture was extracted with CH2Cl2 (3 × 15 ml), evaporated on celite and finally purified by column chromatography (5:95, Et3N:EtOAc). The product was precipitated as the hydrochloride salt (35 mg, 39%). Rf = 0.42% (Et3N in EtOAc); 1H NMR (400 MHz, DMSO-d6) δ 7.42 (2H, m), 7.23 (1H, s), 7.11 (1H, m), 7.04 (2H, m), 4.79 (1H, m), 4.69 (1H, m), 4.27 (1H, m), 4.23 (1H, m), 4.15 (2H, br s), 3.84 (3H, s), 3.77 (3H, s), 3.11 (2H, s br) 2.91 (2H, s br). 13C NMR (100 MHz, DMSO-d6) δ 157.7, 152.0, 148.5, 131.4, 130.9, 125.4, 120.4, 119.6, 117.0, 115.9, 111.2, 109.9, 82.7 (JF-C = 167.6 Hz), 69.1 (JF-C = 19.8 Hz), 56.3, 55.6, 45.9, 45.1 26.2.

2.3.2. Synthesis of 2-(4-bromo-2-(fluoroethoxy)-5-methoxyphenyl)-N-(2-methoxybenzyl)ethan-1-amine oxalate (2)

A solution of tert-butyl 4-bromo-2-hydroxy-5-methoxyphenethyl (2-methoxybenzyl)carbamate 11, (0.466 g, 1.0 mmol) and 2-fluoroethanol (0.096 g, 1.5 mmol) in dry THF (10 ml) was treated with PPh3 (0.393 g, 1.5 mmol) and DIAD (0.303 g, 1.5 mmol) at room temperature for 1 h. The reaction mixture was diluted with EtO (20 ml), filtered and concentrated under reduced pressure. The residue was dissolved in CH2Cl2 (5 ml) and treated with TFA (5 ml) at room temperature for 10 min. The reaction mixture was poured onto ice-water (20 ml) and pH was adjusted to 12 with conc. NaOH. The mixture was extracted with CH2Cl2 (3 × 10 ml) and the combined organic extracts were dried (Na2SO4), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (CHCl3/2 M methanolic NH3 50:1) and the fractions containing the desired compound were concentrated under reduced pressure. The free base was precipitated as the oxalic acid salt by dissolution in diethyl ether and addition of acetic acid dissolved in acetone until complete precipitation of the salt which was isolated by filtration as a white solid. (0.278 g, 62% over two steps). 1H NMR (400 MHz, DMSO-d6) δ 7.40 (2H, m), 7.27 (1H, s), 7.10 (1H, m), 7.01 (2H, m), 4.77 (1H, m), 4.63 (1H, m), 4.28 (1H, m), 4.21 (1H, m), 4.13 (2H, br s), 3.83 (3H, s), 3.80 (3H, s), 3.12 (2H, s br) 2.95 (2H, s br). 13C NMR (100 MHz, DMSO-d6) δ 157.4, 150.4, 149.8, 131.1, 130.7, 126.1, 120.4, 117.4, 114.9, 114.9, 108.9, 83.0 (δF-C = 167.6 Hz), 67.7 (JF-C = 19.8 Hz), 56.3, 55.6, 45.9, 45.1 26.2.

2.3.3. Synthesis of 2-(4-bromo-2,5-dimethoxyphenyl)-N-(2-(2-fluoroethoxy)benzyl)ethan-1-amine (3)

NaH (10 mg, 0.42 mmol) was gradually added to 12 (191 mg, 0.42 mmol) dissolved in 10 ml of dry DMSO and stirred for 30 min. To the resulting mixture, 1,2-dibromoethane (53 mg, 31 μl, 0.42 mmol) was injected slowly and afterwards stirred for 20 h at 60 °C. After evaporation of the solvent, the residue was taken up in TFA/DCM (2 ml) (1:1) and stirred for 8 h at RT. Then the solvent was removed, the residue taken up with EtOAc, washed with NaHCO3 solution and brine and finally extracted with EtOAc (3 × 15 ml). The combined organic extracts were dried (Na2SO4), filtered and evaporated. Chromatography (EtOAc/heptane 1:2) of the residue gave the pure product (117 mg, 0.28 mmol, 68%) as a white semi-solid.

1H NMR (400 MHz, DMSO-d6) δ 7.51 (1H, m), 7.40 (2H, m), 7.39 (1H, m), 7.19 (1H, s), 7.11 (1H, m), 7.01 (2H, m), 4.87 (1H, m), 4.73 (1H, m), 4.36 (1H, m), 4.29 (1H, m), 4.14 (2H, br s), 3.79 (3H, s), 3.74 (3H, s), 3.60 (2H, m) 2.97 (2H, m). 13C NMR (100 MHz, DMSO-d6) δ 156.5, 151.5, 149.4, 131.5, 130.7, 124.5, 120.9, 120.0, 115.9, 114.9, 111.3, 108.9, 83.0 (δF-C = 166.5 Hz), 67.7 (JF-C = 19.1 Hz), 56.6, 55.5, 46.0, 45.2 26.6.

2.4. [18F]Cimbi-36 binding assay

Comounds used for pharmacological testing were more than 99% pure based on NMR and LC-MS. The binding affinities of the three ligands for the 5-HT2A and human 5-HT2C receptors were determined at 37 °C, pH 7.4, in a fluorescence-based Ca2+ imaging assay using Fluo-4 dye. Cells were split into poly-D-lysine-coated black 96-well plates with clear bottom. The following day, the culture medium was aspirated and the cells were incubated in 50 μl of assay buffer [Hank's Buffered Saline Solution containing 20 mM HEPES, 1 mM CaCl2, 1 mM MgCl2, and 2.5 mM propranolol, pH 7.4] supplemented with 6 mM Fluo — 4/AM at 37 °C for 1 h. Then the buffer was aspirated, the cells were washed once with 100 μl of assay buffer, after which 100 μl assay buffer was added to the cells. The 96-well plate was assayed in a FLEXStation 3 (Molecular Devices, Crawley, UK) measuring emission [in fluorescence units (FU)] at 525 nm caused by excitation at 485 nm and after addition of 33.3 μl of agonist solution in assay buffer.

2.5. Ca2+ /Fluo-4 assay

The functional properties of the three ligands at the h5-HT2A and h5-HT2C receptors were determined at stable HEK293 cell lines expressing the two receptors [22] in a fluorescence-based Ca2+ imaging assay using Fluo-4 dye. Cells were split into poly-D-lysine-coated black 96-well plates with clear bottom. The following day, the culture medium was aspirated and the cells were incubated in 50 μl of assay buffer [Hank's Buffered Saline Solution containing 20 mM HEPES, 1 mM CaCl2, 1 mM MgCl2, and 2.5 mM propranolol, pH 7.4] supplemented with 6 mM Fluo — 4/AM at 37 °C for 1 h. Then the buffer was aspirated, the cells were washed once with 100 μl of assay buffer, after which 100 μl assay buffer was added to the cells. The 96-well plate was assayed in a FLEXStation 3 (Molecular Devices, Crawley, UK) measuring emission [in fluorescence units (FU)] at 525 nm caused by excitation at 485 nm and after addition of 33.3 μl of agonist solution in assay buffer.

2.6. Radiosyntheses

Radiolabelling of [11C]Cimbi-36 was performed as previously described. [14].

2.6.1. Preparation of the [18F]FJF—K222 complex

[18F]Fluoride was produced via a (p,n)-reaction on a CTI Siemens Cyclotron (Righospitalet, Denmark) by irradiation of H3O+ with 11 MeV protons. An anion exchange resin (Sep-Pak Light Waters AcellPlus QMA cartridge) was washed with aqueous 1 M K2CO3 (10 ml) and rinsed with water (20 ml) and CH3CN (10 ml) by helium pressure (1.5—2 bar). Then the aqueous [18F]fluoride solution was passed through this exchange resin and the resin eluted with 0.6 ml of a Kryptofix222/K2CO3 solution (K222: 4,7,13,16,21,24-hexa-1,10-diazacyclo[8.8.8]hexacosane, 10 mg, 25 μl in ace tonitrile plus 15 μl K2CO3 (1 M aq.). The resulting mixture was then gently concentrated to dryness by heating at 90 °C under a nitrogen stream for 15 min to give no-carrier-added [18F]FJF—K222 complex as a white semi-solid residue.

2.6.2. 2-[18F]Fluoroethyltosylate([18F]FETos) synthesis

To the dried K222complex as a K222 complex and [18F]FETos was added by HPLC (Onyx Monolithic C18 column (Phenomenex Inc.) 100 × 10 mm; 50:50 H2O:MeCN; flow
rate of 9 mL/min; retention time [RT]: 10 min). After dilution of the collected HPLC fraction with water (20 mL), this resultant solution was loaded onto a Phenomenex Strata-X 33-μm polymeric reversed-phase SPE cartridge. The cartridge was dried with nitrogen and the product eluted with 1 mL of tempered (40–50 °C) DMSO. The synthesis was typically performed within 75 min in a radiochemical yield of 40–50% determined via HPLC. Typical isolated radioactivity amounts were 3.6 ± 0.5 GBq.

2.6.3. Radiolabeling of Cimbi-36 derivatives: [18F]1, [18F]2 and [18F]3

A solution of [18F]FETos in DMSO (1 ml) was added to a 7 mL vial containing the corresponding precursor (10 mg, 0.02 mmol) and Cs2CO3 (10 mg, 0.030 mmol) followed by heating at 120 °C for 10 min. Subsequently, a solution of TFA (0.75 mL), MeCN (0.125 mL) and H2O (0.125 mL) was added and the resulting mixture was heated at 90 °C for 5 min, then diluted with H2O (3.5 mL) and purified by HPLC (Onyx Monolithic C18 column (Phenomenex Inc.) (100 × 10 mm; 25:75 EtOH: 0.1% phosphoric acid; flow rate of 9 mL/min). The fraction corresponding to the labeled product was collected in a 20 mL vial containing 9 mL phosphate buffered saline. All compounds were produced with a 30 min beam. Retention times: [18F]1: R_t = 400 s; isolated yield 2.01 GBq, [18F]2: R_t = 550 s; isolated yield 0.4 GBq, [18F]3: R_t = 500 s; isolated yield 0.2 GBq.

2.6.4. Determination of specific activity and purity

The radiotracer preparation was visually inspected for clarity, and absence of color and particles. Chemical and radiochemical purities were assessed on the same aliquot by HPLC analysis. Specific activity (A_v) of the radiotracers were calculated from three consecutive HPLC analyses (average) and determined by the area of the UV absorbance peak corresponding to the radiolabeled product on the HPLC chromatogram compared to a standard curve relating mass to UV absorbance (296 nm). Column used: Kinetex 2.6 mm, C18 100 Å column (Phenomenex Inc.) (50 × 4.6 mm) 33:67 MeCN:0.1% phosphoric acid; flow rate: 1.5 mL/min. Retention times: [18F]1: R_c = 1.6 min, A_v = 40 GBq/μmol, [18F]2: R_c = 2.4 min, A_v = 122 GBq/μmol, [18F]3: R_c = 2.9 min, A_v = 64 GBq/μmol ([18F]FETos; R_c = 6.6 min). The radiochemical purity of [18F]3 was 87% due to problems with the HPLC procedure. Since only minor uptake of [18F]3 (or the radiochemical impurity) was seen within the brain (see below), the experiment was not repeated. Radiochemical purity of 1 and 2 was >96%.

2.7. In vivo experiments

2.7.1. Animal procedure

Seven female Danish Landrace pigs (mean weight ± SD, 21 ± 4.7 kg) were used for in vivo PET imaging. 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 (i.m.)) and anesthesia was induced with i.m. 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, anesthesia was maintained with constant propofol infusion (10 mg/kg/h intravenous (i.v.); B. Braun, Melsungen, Germany). Venous access was granted through two catheters in the peripheral milk veins. During anesthesia, animals were endotracheally intubated and ventilated. Vital parameters (heart rate, body temperature, blood pressure, oxygen saturation and end tidal CO2) were continuously monitored during the scan. The pigs were euthanized immediately after scanning with an i.v. injection of pentobarbital. All

Scheme 1. a) DMF, K2CO3, FeI, then DCM, M-CPBA b) MeCN, (CHO)2, MgCl2, Et2N, 16 h reflux c) Me2SO4, K2CO3, DMF, RT 16 h d) MeNO2, NH4OAc, RT 5 h, 7% over 4 steps e) LAH, THF, reflux 30 min, 37% f) Br2, AcOH, 2 h 48% g) 2-hydroxybenzaldehyde, EtOH, Et3N, NaBH4, HC1, 39%.

Scheme 2. a) FeI(OH), PPh3, DIAD, THF, 60 min, RT b) TFA, DCM, 10 min, RT c) NaH, FeBr3, DMSO, 20 h, 60 °C d) TFA, DCM, 8 h, RT.
Fig. 2. Time-activity curves and summed PET images for \(^{[11C]}\)Cimbi-36 (\(n = 4, 529 \pm 38\) MBq) and the three \(^{18F}\)-labeled analogues: \(^{[18F]}\)1 (\(n = 1, 505\) MBq), \(^{[18F]}\)2 (\(n = 1, 131\) MBq) and \(^{[18F]}\)3 (\(n = 1, 133\) MBq, 87% radiochemical purity). Arrows in the TACs represent the time of a ketanserin challenge (90 min post tracer injection) with indicated doses. All PET images are summed over 0-90 min and with a 3 mm Gaussian filtration and are overlaid with a standardized MR-atlas of the pig brain. All animals were anesthetized with propofol (10 mg/kg/h) during the experiments. Error bars represent standard deviation.

Scheme 3. Radiolabeling of \(^{[18F]}\)1, \(^{[18F]}\)2 and \(^{[18F]}\)3: a) \(^{[18F]}\)FETos, Cs\(_2\)CO\(_3\), 10 min, 120 °C b) TFA/MeCN/H\(_2\)O, 5 min.
animal procedures were approved by the Danish Council for Animal Ethics (journal no. 2012–15–2934-00,156).

2.7.2. PET scanning protocol

All tracers were given as i.v. injections. Injected radioactive and mass doses were as follows: [11C]Cimbi-36: 529 ± 38 MBq, 14.0 ± 18.0 μg (n = 4); [18F]F1: 505 MBq, 5.9 μg, n = 1; [18F]F2: 131 MBq, 0.46 μg, n = 1; [18F]F3: 133 MBq, 1.1 μg, n = 1. The pigs were scanned for 90 min with [11C]Cimbi-36 and 150 min for all [18F]-labeled tracers in list mode in a high resolution research tomography (HRRT) scanner (Siemens AG). PET-scanning was started at the time of injection (t = 0 min). Ninety minutes after injection of the [18F]-labeled tracer, ketanserin was given as i.v. injection. In the study with [18F]F1 the dose was 1 mg/kg (bolus) with a subsequent constant infusion of 1 mg/kg/h. In the studies with [18F]F2 and [18F]F3 the bolus dose was 5 mg/kg. Blood samples were drawn at time points 2.5, 5, 10, 20, 30, 40, 50, 60, 70, 89, 91, 120 and 150 min.

2.7.3. Quantification of PET data

The HRRT list mode data were reconstructed and analyzed as previously described [7].

2.7.4. Assessment of radioactive metabolites

The relative fractions of parent tracer and radiometabolites in arterial pig plasma were determined using radio-HPLC. Samples of arterial whole blood (10 mL) drawn during PET acquisition (described above) were centrifuged (1500 × g for 7 min at 5 °C) and the plasma phase was filtered through a syringe filter (Whatman GD/X filter with 25 nm PVDF membrane). Analysis by radio-HPLC was performed by direct injection of up to 4 mL of plasma to a restricted access material (25 mm PVDF membrane). Analysis by radio-HPLC was performed by direct injection of up to 4 mL of plasma to a restricted access material (25 mm PVDF membrane). Analysis by radio-HPLC was performed by direct injection of up to 4 mL of plasma to a restricted access material (25 mm PVDF membrane).

PET data were reconstructed by HRRT and PET data were analyzed and visualized using a dedicated software (Osirix, Asellus Software). The binding affinities of the reference compounds were determined at human 5-HT2A and 5-HT2C receptors using [3H]Cimbi-36 as radioligand (Table 1). The substitution of a methoxy-group by a fluoroethoxy group did not alter the affinity of the ligands towards h5-HT2A and h5-HT2C significantly. They remain within one order of magnitude relative to the parent compound, Cimbi-36, with similar 2A/2C selectivity profiles. The Ks values of Cimbi-36 at h5-HT2AR and h5-HT2CR were previously reported to be 0.5 and 1.7 nM, respectively (~3 fold selective) using [3H]Ketanserin as radioligand [14], but the use of [18F]Cimbi-36 should give a more accurate representation of the relative affinities of the compounds, as they are from the same compound class. Furthermore, a functional assay revealed that the three compounds were potent partial agonists at the human 5-HT2A and 5-HT2C receptors with EC50 values in the low nM range (Table 2).

Encouraged by their promising in vitro profiles, we proceeded with the development of the 18F-labeling of all three compounds and subsequently evaluated these PET tracers in Danish Landrace pigs.

Radiolabeling with [18F]FETos yielded 0.2–2.1 GBq of formulated product (Scheme 3). The final formulation of the injectable solution including two preparative HPLCs purifications steps (purification of [18F]FETos and the final product) took no longer than 100 min for all compounds and provided [18F]-labeled tracers with a radiochemical purity of >96% (except [18F]F3 which was obtained in 87% purity) and chemical purity of >98% as determined by HPLC analyses. Typical specific activities (A2) were between 40 and 120 GBq/μmol.

After intravenous injection, [18F]F1 (n = 1) showed a high brain uptake, similar to [11C]Cimbi-36 (Fig. 2). In contrast to [11C]Cimbi-36, there was only a small difference between the uptake in cortex, a high binding region, and the uptake in cerebellum, a low binding region. Administration of the 5-HT2AR antagonist ketanserin during the scan did not result in any appreciable decrease in binding in any brain regions, suggesting limited or no specific binding of [18F]F1. In addition, [18F]F1 uptake was significantly higher than that seen with [11C]Cimbi-36 in the cerebellum and cortex. PET data were analyzed using a dedicated software (Osirix, Asellus Software).

Table 2

<table>
<thead>
<tr>
<th>Compound</th>
<th>Receptor</th>
<th>EC50 [pEC50 ± S.E.M.]</th>
<th>n1 ± S.E.M.</th>
<th>Rmax ± S.E.M.</th>
<th>2A/2C-selectivity (EC50/EC250)</th>
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<td>Cimbi-36</td>
<td>h5-HT2A</td>
<td>0.95 [9.02 ± 0.09] (4)</td>
<td>1.12 ± 0.11</td>
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<td>0.90 [9.04 ± 0.06] (4)</td>
<td>1.22 ± 0.07</td>
<td>99 ± 7</td>
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<td>4.1 [3.83 ± 0.12] (3)</td>
<td>1.19 ± 0.14</td>
<td>72 ± 4</td>
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</tr>
<tr>
<td></td>
<td>h5-HT2C</td>
<td>12 [7.91 ± 0.08] (5)</td>
<td>1.23 ± 0.06</td>
<td>75 ± 3</td>
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<tr>
<td>2</td>
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<td>2.0 [8.71 ± 0.14] (4)</td>
<td>0.95 ± 0.07</td>
<td>77 ± 5</td>
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<td>h5-HT2C</td>
<td>1.9 [8.72 ± 0.09] (4)</td>
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<td>3</td>
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<td>7.4 [8.13 ± 0.08] (5)</td>
<td>1.29 ± 0.12</td>
<td>51 ± 4</td>
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<tr>
<td></td>
<td>h5-HT2C</td>
<td>4.6 [8.34 ± 0.07] (5)</td>
<td>1.07 ± 0.11</td>
<td>61 ± 3</td>
<td>0.6</td>
</tr>
</tbody>
</table>

EC50 values are given in nM with pEC50 ± S.E.M. values in brackets. Hill slopes (nH) are given with S.E.M. values, and Rmax ± S.E.M. values are given in % of the Rmax obtained for 5-HT at the receptor at the same plate. The number of experiments (n) is given in parenthesis after each EC50 value.
also showed signs of defluorination, which is seen by a pronounced bone uptake in the skull (Fig. 2). Although it appears that the ratio between cortex and cerebellum reduces after the ketanserin challenge, this effect is merely a partial volume effect from the skull surrounding the cerebellum, leading to the counts in the cerebellum reaching a constant level as the bone uptake increases over the duration of the scan. Furthermore, we have recently elucidated that for [11C]Cimbi-36 and other related NBOMe's, the major metabolic pathway is O-dealkylation at the 5′-position followed by glucuronidation [25]. Assuming a similar metabolic pathway for [18F]1, a major radiometabolite will be [18F]fluoroacetate. It is well known that [18F]fluoroacetate and its metabolites ([18F]fluoroethanol and [18F]fluoroacetate) readily cross the blood brain barrier and are accumulated in brain [26–28]. Based on that we suggest that a large proportion of the radioactivity seen in brain following administration of [18F]1 can be attributed to the formation of [18F]fluoroacetdehyde and its metabolites. In the case of [18F]2 and [18F]3 very low brain uptake was observed with negligible separation between cortex and cerebellum. The radiochemical purity of [18F]3 was only 97%, but given the negative result we decided not to repeat the experiment as this would require us to sacrifice another animal.

Approximately 15–20% of the parent compounds [18F]1, [18F]2 and [18F]3 remained intact in plasma after 30 min (Fig. 3). This indicates that the poor brain penetration of the ligands cannot be attributed to rapid metabolism and suggests that they cannot cross the blood–brain-barrier (BBB) to any major extent or that they are rapidly removed by efflux pumps.

We observed a slightly increased plasma concentration of [18F]1, [18F]2 and [18F]3 after ketanserin administration at 90 min (see Fig. 2), suggesting that the radioligands bind to peripheral sites wherefrom they can be displaced by ketanserin.

4. Conclusion

We successfully [18F]-labeled three Cimbi-36 derivatives by sequentially replacing three different methoxy groups with a [18F]ethyl-moiety and evaluated them in Danish Landrace pigs. Although for [18F]1 there was some brain uptake, we suggest that a large proportion of the radioactivity in the brain was due to radiometabolites, which would explain why it could not be displaced with a competitive 5-HT2A antagonist. In summary, we have synthesized and tested three [18F]-labeled agonist PET tracers but none of them appears to be suitable candidates for functional neuroimaging of 5-HT2A-Rs in vivo.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.nucmedbio.2016.02.011.

References


