Bioorganic & Medicinal Chemistry 21 (2013) 5598–5604

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis and in vivo evaluation of [^{18}F]2-(4-(4-(2-(2-fluoroethoxy)phenyl)piperazin-1-yl)butyl)-4-methyl-1,2,4-triazine-3,5(2*H*,4*H*)-dione ([^{18}F]FECUMI-101) as an imaging probe for 5-HT$_{1A}$ receptor agonist in nonhuman primates

Vattoly J. Majo [a], Matthew S. Milak [a,b], Jaya Prabhakaran [a], Pratap Mali [b], Lyudmila Savenkova [a], Norman R. Simpson [b], J. John Mann [a,b,c], Ramin V. Parsey [d], J. S. Dileep Kumar [a,b,*]

[a] Department of Psychiatry, Columbia University Medical Center, Columbia University, 1051 Riverside Drive, Box #42, New York, NY 10032, United States
[b] Division of Molecular Imaging and Neuropathology, New York State Psychiatric Institute, New York, United States
[c] Department of Radiology, Columbia University, New York, NY 10032, United States
[d] Department of Psychiatry and Behavioral Science, Stony Brook University, New York, United States

ARTICLE INFO

Article history:
Received 12 March 2013
Revised 9 May 2013
Accepted 17 May 2013
Available online 12 June 2013

Keywords:
PET
FECUMI-101
5-HT1AR
Agonist
Brain
Radiotracer

ABSTRACT

The 5-HT$_{1A}$R partial agonist PET radiotracer, [^{11}C]CUMI-101, has advantages over an antagonist radiotracer as it binds preferentially to the high affinity state of the receptor and thereby provides more functionally meaningful information. The major drawback of C-11 tracers is the lack of cyclotron facility in many health care centers thereby limiting widespread clinical or research use. We identified the fluoroethyl derivative, 2-(4-(4-(2-(2-fluoroethoxy)phenyl)piperazin-1-yl)butyl)-4-methyl-1,2,4-triazine-3,5(2*H*,4*H*)dione (FECUMI-101) (K_i = 0.1 nM; E_{max} = 77%; EC_{50} = 0.65 nM) as a partial agonist 5-HT$_{1A}$R ligand of the parent ligand CUMI-101. FECUMI-101 is radiolabeled with F-18 by *O*-fluoroethylation of the corresponding desmethyl analogue (**1**) with [^{18}F]fluoroethyltosylate in DMSO in the presence of 1.6 equiv of K_2CO_3 in 45 ± 5% yield (EOS). PET shows [^{18}F]FECUMI-101 binds specifically to 5-HT$_{1A}$R enriched brain regions of baboon. The specificity of [^{18}F]FECUMI-101 binding to 5-HT$_{1A}$R was confirmed by challenge studies with the known 5-HT$_{1A}$R ligand WAY100635. These findings indicate that [^{18}F]FECUMI-101 can be a viable agonist ligand for the in vivo quantification of high affinity 5-HT$_{1A}$R with PET.

1. Introduction

The serotonin 1A receptor (5-HT$_{1A}$R) is a G protein-coupled receptor (GPCR) coupled to G_i/G_o.[1] 5-HT$_{1A}$R is both a somatodendritic autoreceptor on serotonin neurons in the midbrain and pons, and a widespread and heterogeneously distributed postsynaptic receptor in the terminal fields.[2] Somatodendritic 5-HT$_{1A}$R autoreceptors are present on serotonergic neurons in brainstem raphe nuclei and inhibit serotonin neuron firing after 5-HT release from recurrent fibers. 5-HT$_{1A}$R heteroreceptors modulate or regulate release of other neurotransmitters, proteins, hormones and growth factors in brain.[3–5] 5-HT$_{1A}$R play a role in cognition, learning, memory, aggression, sociability, impulsivity, addictive behavior, sexual behavior, food intake or appetite, prolongation of rapid eye movement (REM), sleep latency, regulation of respiration, emesis and analgesia.[6] 5-HT$_{1A}$R partial agonists (e.g., buspirone and tandospirone) are currently approved drugs for anxiety and depression.[7,8] Some atypical antipsychotics like aripiprazole are also partial agonists at the 5-HT$_{1A}$R and used as antidepressants.[9,10] 5-HT$_{1A}$R desensitization and increased serotonin neuron firing is observed in animal studies and hypothesized to be part of the mechanism of antidepressant action of selective serotonin reuptake inhibitors (SSRIs), serotonin-norepinephrine reuptake inhibitors (SNRIs), tricyclic antidepressants (TCAs), tetracyclic antidepressants (TeCAs), and monoamine oxidase inhibitors (MAOIs).[3–7]

Quantitative imaging of the serotonergic system is of critical importance for understanding the pathophysiology of psychiatric and neurological illnesses.[11–13,17–21] Positron Emission Tomography (PET) has the sensitivity to measure 5-HT$_{1A}$Rs in living brain, and antagonist PET ligands (e.g., [^{11}C]WAY-100635, [^{18}F]MPPF, [^{11}C]FCWAY and [^{18}F]MeFWAY) are the commonly used radiotracers for imaging 5-HT$_{1A}$Rs[14–17]. However, 5-HT$_{1A}$R antagonist ligands bind with equal affinity to high agonist affinity (HA) and low agonist affinity (LA) sites of GPCRs, and cannot detect changes or differences in HA sites that are only a proportion of the total number of receptors.[22] In contrast, agonist ligands preferentially bind to the HA receptor conformation thereby providing a more meaningful functional measure of 5-HT$_{1A}$R transduction

* Corresponding author. Tel.: +1 (212) 543 1163; fax: +1 (212) 543 1054.
E-mail address: kumardi@nyspi.columbia.edu (J. S. Dileep Kumar).

0968-0896/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmc.2013.05.050

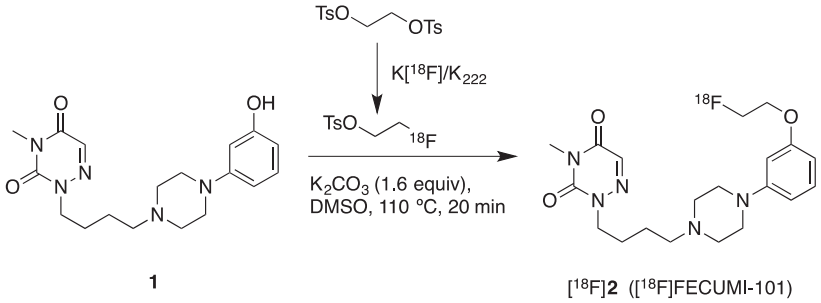
capacity.[22] The ratio of [^{3}H]WAY100,635: [^{3}H]8-OH-DPAT or antagonist: agonist receptor binding in human neocortex is 1.7–2.7 across different brain regions, using quantitative autoradiography).[23] In rat hippocampal tissue, the LA: HA ratio average is ~1.5.[22] Alterations in the HA affinity site of 5-HT$_{1A}$R receptors may be an important factor in the pathophysiology of diseases, and is part of the hypothesized action of many antidepressants. Therefore, obtaining PET scans with an agonist ligand in the same subject would enable the quantification of binding by GPCRs, that, in turn, is related to the level of signal transduction by endogenous 5-HT. Moreover, an agonist 5-HT$_{1A}$R radiotracer is likely to be sensitive to changes in intra-synaptic concentrations of the endogenous serotonin, measures desensitization (downregulation) or sensitization (upregulation) of GPCRs, provides a better estimate of receptor occupancy for agonist therapeutic agents and for evaluation of the efficacy of SSRI treatment.[24–30] The translocator protein 18 kDa (TSPO) radioligand [^{18}F]DPA-714, κ-Opioid receptor radioligand [^{11}C]GR103545, D_2/D_3 radiotracers [^{11}C]PHNO and [^{11}C]NPA, 5-HT$_{2A}$ radioligand [^{11}C]CIMBI-36 are some of the agonist PET tracers that appears to be superior to the corresponding antagonist tracers in vivo.[31–35] [^{11}C]CUMI-101, developed by us, is the only successful partial agonist radiotracer that binds to the HA state of 5-HT$_{1A}$R in nonhuman primates and human subjects.[36–39] We have also demonstrated the ability of [^{11}C]CUMI-101 to measure robust increases in intrasynaptic endogenous serotonin in baboons by iv citalopram and fenfluramine administration.[40,41] As predicted, lower BP_F was observed for [^{11}C]CUMI-101 scans in comparison with [^{11}C]WAY-100635 scans (~50%) for 5-HT$_{1A}$R—rich regions such as hippocampus, amygdala, cortex and cingulate regions.[42] These findings are consistent with autoradiography studies using tritiated ligands 8-OH-DPAT and WAY100635.[22,23] Although [^{11}C]CUMI-101 is a promising radiotracer in human, its major limitation in serving clinical populations is the requirement for an onsite cyclotron to produce C-11. However, F-18 tracer can be shipped to multiple medical centers that are within a radius of 4 h transportation time from the production site and the 110-min half-life of F-18 labeled agonist tracer also permits imaging for a longer duration facilitating tracer kinetic modeling studies. Moreover, the F-18 positron energy is the lowest of the first row positron emitters and imaging can be potentially done at the highest resolution[45–47]. Hence development of an F-18 labeled 5-HT$_{1A}$R agonist tracer may have many advantages. We have developed several fluoro analogues of various 5-HT$_{1A}$R agonists as PET ligands with limited success. However, 2-(4-(4-(2-(2-fluoro-ethoxy)phenyl)piperazin-1-yl)butyl)-4-methyl-1,2,4-triazine-3,5(2*H*,4*H*)-dione (FECUMI-101), the fluoroethyl analogue of CUMI-101 shows promising characteristics as a 5-HT$_{1A}$R imaging agent. Herein we describe radiosynthesis and in vivo evaluation of [^{18}F]FECUMI-101 in nonhuman primates with PET (Scheme 1).

2. Results

2.1. Chemistry, pharmacology and radiochemistry

The reference standard FECUMI-101 was synthesized by heating desmethyl-CUMI-101 (**1**) with bromoethyl fluoride (1.5 equiv) for 4 h at 80 °C in the presence of excess K_2CO_3 (5 equiv) in DMF in 65% yield. Synthesis of **1** was achieved using a procedure developed by us.[36] The affinity of FECUMI-101 for 5-HT$_{1A}$R and various other biogenic amine receptors and transporters and functional assays were determined through the National Institute of Mental Health Psychoactive Drug Screening Program (NIMH-PDSP). Binding assay shows that FECUMI-101 has 0.1 nM binding affinity (K_i) for 5-HT$_{1A}$R. FECUMI-101 has a K_i of 21.4 ± 0.6 nM for alpha$_{1A}$R and exhibits nanomolar affinities for 5-HT$_7$R (K_i = 17.2 nM), D_2R (K_i = 37.1 nM), D_3R (K_i = 22 nM) and D_4R (K_i = 71 nM) receptors (Table 1). The K_is for various other brain receptors and transporters were low (0.1–10 μM). Agonist properties of FECUMI-101 on 5-HT$_{1A}$R were evaluated using [^{35}S]GTPγS formation in membranes of CHO cells stably expressing the human 5-HT$_{1A}$R. FECUMI-101 produced a dose-dependent increase in [^{35}S]GTPγS binding. Maximal FECUMI-101 stimulated [^{35}S]GTPγS binding for 5-HT$_{1A}$R was 77% that of serotonin (E_{max}) with an EC_{50} of 0.65 nM. The affinity, cross selectivity and functional properties of CUMI-101 and FECUMI-101 are presented in Table 1.

Radiosynthesis of [^{18}F]FECUMI-101 was performed by a two-step fluroalkylation of the phenolate of **1** (Scheme 1). For this purpose, initially we synthesized [^{18}F]fluoroethyltosylate by radioflourination of ethyleneglycol ditosylate with K[^{18}F], kryptofix K_{222} and K_2CO_3 in 90% crude yield.[43] [^{18}F]fluoroethyltosylate ([^{18}F]FETos) was then purified via semiprep RP HPLC (75%). When LiChrolut EN column was used instead of C-18 Sep-Pak® to trap [^{18}F]FETos, a 15% better efficiency was observed. [^{18}F]FETos reacted with **1** in the presence of a base to afford [^{18}F]FECUMI-101 ([^{18}F]**2**). The starting material was unreacted in acetonitrile at temperatures below 100 °C. When aqueous NaOH was used as the base, [^{18}F]FCH_2CH_2OTs decomposed at temperatures above 125 °C. The anhydrous sodium salt of precursor obtained by azeotropic distillation of precursor with 5 M NaOH (1.8 equiv) in acetonitrile reacted with [^{18}F]FCH_2CH_2OTs in DMSO at 110 °C to yield [^{18}F]**2** in 25% yield (EOS). Better yields were obtained by using 1.6 equiv of aqueous K_2CO_3 (25 μL, 5%) as a base at 110 °C. The crude product was purified by RP-HPLC followed by C-18 Sep-Pak® purification to obtain [^{18}F]FECUMI-101 in 45 ± 5% yield at EOS (n = 10). Specific activity obtained for [^{18}F]FECUMI-101 was 92.5 ± 18.5 GBq/μmol (n = 10) based on a standard mass curve with >95% chemical and radiochemical purities. The total time required for the radiolabeling process was 60 min at EOS. A one-pot modification of the procedure was later developed to provide [^{18}F]FECUMI-101, albeit, in slightly lower yields (20–25%). The log$P_{o/w}$ of [^{18}F]**2** from shake



Scheme 1. Radiosynthesis of [^{18}F]FECUMI-101.

Table 1
Comparison of binding affinities and GTPγ functional assay results of FECUMI-101 and CUMI-101

Binding assays	K_i (nM)	
	FECUMI-101	CUMI-101
$5HT_{1A}$	0.1	0.15
$5HT_{1B}$	411	643
$5HT_{1D}$	234.6	88.3
$5HT_{2A}$	>10,000	4,975
$5HT_{2B}$	300	73.6
$5HT_{5A}$	2128	>10,000
$5HT_7$	17.2	12.9
Adrenergic α_{1A}	21.4	6.75
Adrenergic α_{1B}	26.8	>10,000
Adrenergic α_{1D}	62.6	>10,000
Adrenergic α_{2A}	2751	96
Adrenergic α_{2B}	56.2	238
Adrenergic α_{2C}	173.7	15
BZP rat brain site	6238	>10,000
Dopamine1	>10,000	>10,000
Dopamine2	37.1	>10,000
Dopamine3	22	>10,000
Dopamine4	71	21
Histamine1	512	1030
Histamine2	1767	2126
Sigma 1	>10,000	59
Sigma 2	1726	123
Agonist assay (GTP-γS)		
E_{max}	77%	80%
EC_{50}	0.65 nM	0.1 nM

flask method was 1.2.[44] Stability of [^{18}F]**2** formulation (10% ethanol-90% saline) used for in vivo studies was analyzed using analytical HPLC and radio-TLC and indicated that the radioproduct was stable for up to 4 h with no significant de[^{18}F]fluorination in the formulation.

2.2. Determination of unchanged radioligand in plasma

Figure 1 shows the time course of the unmetabolized fraction of [^{18}F]FECUMI-101 in baboon plasma. [^{18}F]FECUMI-101 showed 35 ± 1.5% (n = 12) and 11.5 ± 2.5% (n = 12) binding in standardized baboon and human samples respectively. HPLC analyses of the plasma samples indicated only polar metabolites and the percentage of unmetabolized [^{18}F]FECUMI-101 was 98 ± 1.0% at 2 min, 74 ± 2.5% at 12 min, 51% at 30 min, 37 ± 4.0% at 60 min, 32.5 ± 0.5% at 90 min, 23.5 ± 2% at 120 min, 21.5 ± 2% at 150 min and 18 ± 2% at 180 min, respectively.

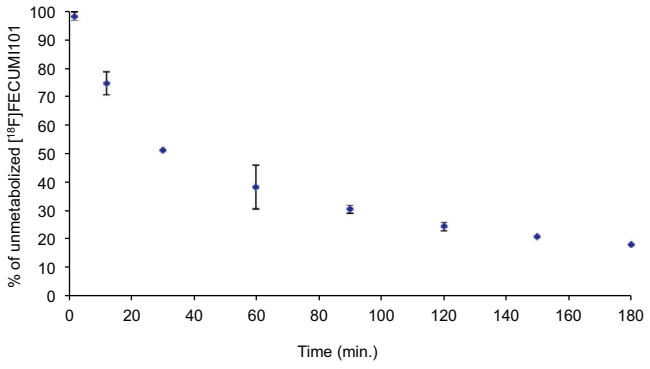


Figure 1. Unmetabolized parent fraction of [^{18}F]FECUMI-101 in baboon plasma.

2.3. PET imaging studies with [^{18}F]FECUMI-101 in baboon

PET studies in anesthetized baboon (*Papio anubis*) showed that [^{18}F]FECUMI-101 (111 ± 18 MBq) penetrated the BBB and was retained in 5-HT_{1A}R rich areas such as hippocampus, insula, cingulate cortex, prefrontal cortex, and amygdala, whereas the striatum exhibited lower binding and cerebellum had the least amount of binding (Fig. 2). No skull image due to in vivo radio[^{18}F]defluorination was observed. The time activity curves (TACs) show radioactivity in all regions reached a peak by 3–13 min post injection and a rapid clearance was observed for cerebellum. The binding ratios of hippocampus, anterior cingulate cortex, insular cortex, cingulate cortex, prefrontal cortex, amygdala, and dorsal raphe nucleus to cerebellum were 4.0, 2.5, 2.3, 1.8, 1.8, 2.4 and 1.75 at 175 min. Other cortical regions with known 5-HT_{1A}R binding show ~twofold binding in comparison to cerebellum. Surprisingly, thalamus showed moderate binding with [^{18}F]FECUMI-101. The binding ratio of thalamus to cerebellum was 2.0 at 175 min. TACs show that radioligand reached equilibrium in 40 min, hence 120 min scan will be suitable for baboon PET studies with [^{18}F]FECUMI-101 (Fig. 3). The specificity of the radioligand uptake was determined by chase studies by the administration of 5-HT_{1A}R antagonist WAY-100,635 (0.5 mg/kg/i.v) 50–60 min after the injection of [^{18}F]FECUMI-101 (Figs. 2 and 4). No significant changes in metabolism or protein binding were observed in chase challenge administration studies with [^{18}F]FECUMI-101. The blocking study showed displacement of radioactivity in all regions close to cerebellar level.

3. Discussion

Alteration of serotonin 1A receptor (5-HT_{1A}R) binding has been implicated in a number of neuropsychiatric and neurodegenerative disorders. It is the HA site that binds to g proteins and is responsible for signal transduction. Hence imaging using an agonist PET tracer is required to measure 5-HT_{1A}R in vivo. [^{11}C]WAY100,635, has been widely used for in vivo quantification of 5-HT_{1A}R with PET. One of the major impediments to using this technology in clinics is the limited number of centers that can produce C-11 labeled radiotracers. We identified [^{11}C]CUMI-101 as an agonist PET tracer for imaging HA 5-HT_{1A}Rs in human and nonhuman primates.[36–42]

We identified FECUMI-101 as a candidate agonist PET tracer through structure activity relationship studies of CUMI-101. FECUMI-101 has comparable binding affinity and agonist properties for 5-HT_{1A}R (K_i = 0.1 nM; E_{max} = 77%, EC_{50} = 0.65 nM) to those of the parent ligand CUMI-101 (K_i = 0.15 nM; E_{max} = 80%, EC_{50} = 0.1 nM) (Table 1). FECUMI-101 was also found to be highly selective for 5-HT_{1A}R over the tested panel of brain biogenic amine receptors, transporters and proteins that were tested through NIMH PDSP. Both FECUMI-101 and CUMI-101 displayed comparable affinities for most of the target receptors and transporters. Some advantages of FECUMI-101 versus CUMI-101 is its lower binding to alpha$_{1A}$R (K_i = 6.75 nM) and sigma1R (K_i = >10,000 nM) in comparison to a K_i of 21.4 and 59 nM, respectively for CUMI-101. However, FECUMI-101 has K_is of 37.1 and 22 nM for D_2R and D_3Rs, whereas CUMI-101 did not show any significant binding to these receptors. However, despite its relatively higher affinity for D_2R and D_3Rs, negligible binding was observed in the striatum and therefore, the excellent affinity, selectivity and agonistic properties of FECUMI-101 for 5-HT_{1A}R and the availability of F-18 labeling site makes it a promising PET ligand for 5-HT_{1A}R.

The tethering of [^{18}F]fluoroethyl group to phenolate of desmethyl-CUMI-101 was achieved in 45 ± 5% yield (EOS) with excellent chemical, radiochemical purities and specific activity (Scheme 1). The total time required for the radiosynthesis was 60 min at EOS.

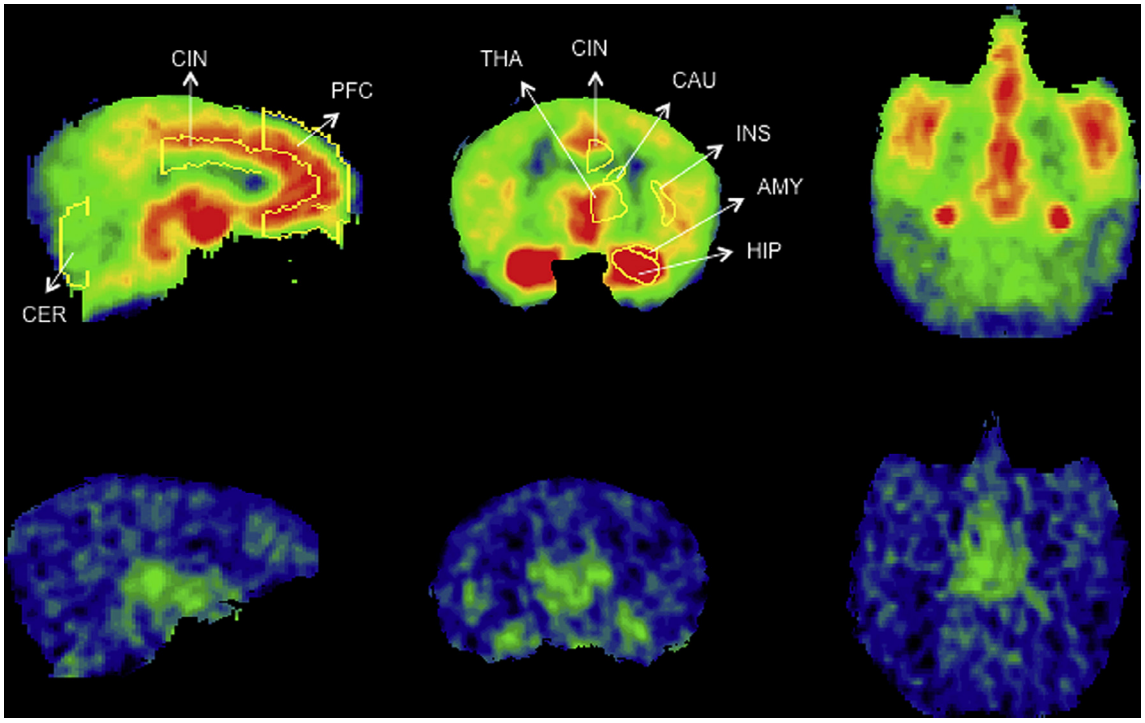


Figure 2. Sum of 60–180 min of [^{18}F]FECUMI-101 image in baboon. 1st row: Base line; 2nd row: Chase with WAY100635. First column: sagittal, middle column: coronal, last column: axial views.

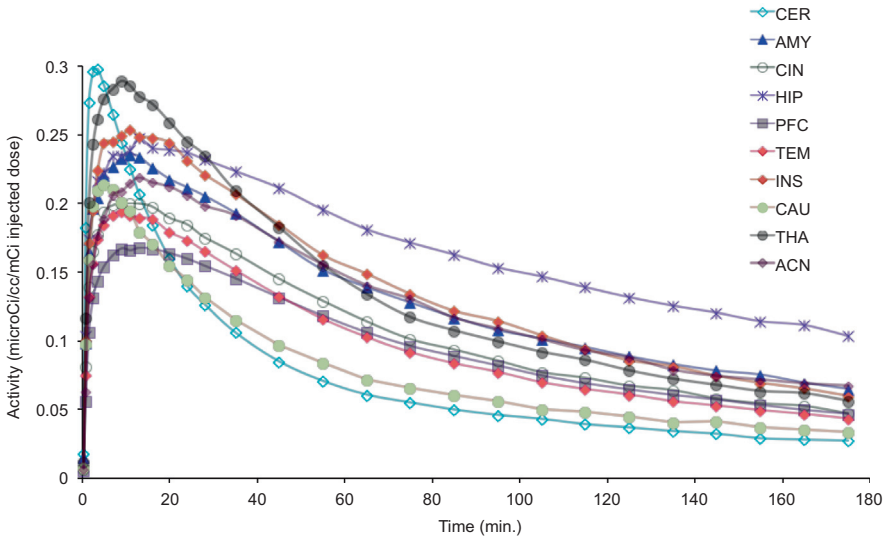


Figure 3. Baseline time activity curves of [^{18}F]FECUMI-101 in baboon. AMY = Amygdala, CAU = Caudate, CER = cerebellum, CIN = cingulate, HIP = hippocampus, PFC = prefrontal cortex, TEM: temporal cortex, INS: insular cortex, THA: thalamus, ACN: anterior cingulate

The lipophilicity value of 1.2 ± 0.5, measured in terms of log$P_{o/w}$, is favorable for BBB penetration of [^{18}F]FECUMI-101. Radiolabeled metabolites found in baboon plasma during PET scan were analyzed using a reverse phase HPLC column coupled with γ-detector. Only polar metabolites were detected in the analyses and these metabolites are unlikely to cross the BBB because of their high polarity and hence the image obtained could be attributed to the parent [^{18}F]FECUMI-101. The radioligand also has a measurable free fraction and this will enable receptor quantification using free fraction corrected arterial input functions. Distribution of [^{18}F]FECUMI-101 binding in baboon brain corresponds to the known distribution of 5-HT$_{1A}$R for human and nonhuman primates.[36,42,48] Highest uptake of [^{18}F]FECUMI-101 radioactivity in baboon was found in hippocampus, anterior cingulate cortex, insular cortex and amygdala. This is consistent with our previous reports of [^{11}C]CUMI-101 binding in baboon and human.[37–40] Dorsal raphe nucleus, temporal cortex, parahippocampal gyrus, pareital cortex, and prefrontal cortex show moderate binding of radioligand. Consistent with previous reports for 5-HT$_{1A}$R PET tracers, striatum and occipital cortex regions show lower binding, whereas, cerebellum shows the least binding of the radiolgand.[36,42,48] To our surprise thalamus shows higher binding of [^{18}F]FECUMI-101 in comparison to [^{11}C]CUMI-101 and [^{11}C]WAY100635. The specificity of the [^{18}F]FECUMI-101 binding was determined via a chase study with

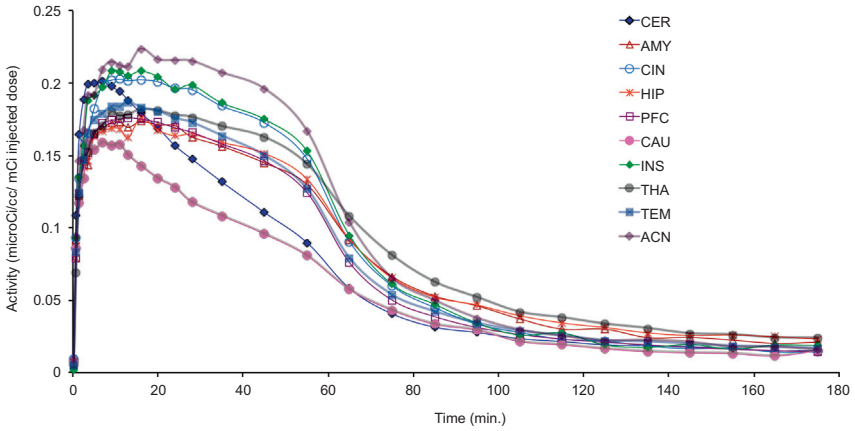


Figure 4. Time activity curves of [^{18}F]FECUMI-101 in baboon after WAY100635 chase.

WAY100635 (0.5 mg/kg iv) and we obtained excellent blockade of radioligand binding throughout the brain including thalamus. The administration of WAY100635 was performed at a time point closer to the time frame in which radioligand reached equilibrium as evident from baseline TACs (Fig. 3).

We believe radioligand binding to thalamus is not due to its 5-HT_{1A}R binding as it is well established that thalamus has low 5-HT_{1A}R concentration. Since the displaceable PET signal consists of density multiplied by affinity, a high B_{max} and high K_d will give a large PET signal compared to the corresponding signal from a relatively low abundance receptor. Receptor distribution (B_{max}) of colocalized targets 5-HT_{1A} and 5-HT_7 in thalamus are reported as 4.16 and 12 fmol/mg of tissue, respectively.[49] Therefore, corresponding ratios of 5-HT_7 to 5-HT_{1A} receptor in thalamus is 2.88. In order to avoid more than 10% PET signal interference from the 5-HT_7 receptors in thalamus, a 29-fold K_d difference over the 5-HT_7 receptor is sufficient. Since FECUMI-101 is 172-fold selective to 5-HT_{1A} over 5-HT_7, no contribution to the PET signal is expected from 5-HT_7R in thalamus. Radioligand binding in striatum is very low, ruling out the possibilities of D_2R and D_3R binding by [^{18}F]FECUMI-101.[50,51]The ratios of FECUMI-101 binding to 5-HT1AR in comparison with adrenergic α_1 receptor (α_1 AR) sub types ranges from 210 to 620 with K_i values 21–62 nM. In the case of in vivo studies with CUMI-101, we did not find much binding of [^{11}C]CUMI-101 in high α_1 AR regions, despite its 6.45 nM K_i for the α_{1A} AR, which is 45-fold less affinity in comparison to 5-HT_{1A}R. For example, there is low binding of [^{18}F]FECUMI-101 in cerebellum and striatum areas with moderate α_1 ARs.[52–54]

The challenge doses of WAY100635 we used were 0.5 mg/kg based on our previous experience[36,48] that this dose would provide substantial blockade of 5-HT_{1A}R. Although WAY100635 is a gold standard ligand for 5-HT_{1A}R, it has considerable affinity for several other receptors.[55] Apart from the thalamic binding, [^{18}F]FECUMI-101 brain binding distribution corresponds to that of 5-HT_{1A}R. Further studies are required to establish the target responsible for the thalamic binding of [^{18}F]FECUMI-101.

4. Materials and methods

4.1. General

The commercial chemicals and solvents used in the synthesis were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO), Fisher Scientific Inc. (Springfield, NJ), or Lancaster (Windham, NH) and were used without further purification. Analytical grade reagents were purchased from standard commercial sources. ^{1}H NMR spectra were recorded on a Bruker PPX 300 and 400 MHz spectrometer. Spectra were recorded in $CDCl_3$ or CD_3OD and chemical shifts and reported in ppm relative to TMS as internal standard. The mass spectra were recorded on JKS-HX 11UHF/HX110 HF Tandem Mass Spectrometer in the FAB+ mode. Thin layer chromatography (TLC) was performed using silica gel 60 F254 plates from E Merck. HPLC analyses were performed using a Waters 1525 binary HPLC system (analytical: Phenomenex, Prodigy ODS(3) 4.6 × 250 mm, 5 μ column; semipreparative: Phenomenex, Prodigy ODS-prep 10 × 250 mm, 10 μ column). Flash column chromatography was performed on silica gel (Fisher 200–400 mesh) using the solvent system indicated. [^{18}F]fluoride was produced from an RDS112 cyclotron (Siemens, Knoxville, TN). For detection of radiolabeled [^{18}F]FECUMI-101, gamma ray detector (Bioscan Flow-Count fitted with a NaI detector) was used in series with the UV detector (Waters Model 996 set at 254 nm). Data acquisition for both the analytical and preparative systems was accomplished using a Waters Empower Chromatography System. PET studies were performed in baboon using an ECAT EXACT HR+ scanner (Siemens, Knoxville, TN). All animal experiments were carried out with the approval of the Institutional Animal Care and Use Committee of Columbia University Medical Center and New York State Psychiatric Institute. Metabolite analyses were performed using Phenomenex Prodigy column (ODS3, 4.6 × 250 mm, 5 μ). The free fractions and metabolites were measured using Packard Instruments Gamma Counter (Model E5005, Downers Grove, IL).

4.2. Synthesis of 2-(4-(4-(2-(2-fluoroethoxy)phenyl)piperazin-1-yl)butyl)-4-methyl-1,2,4-triazine-3,5(2*H*,4*H*)-dione (2)

1-Bromo-2-fluoroethane (26.4 mg, 0.208 mmol) and potassium carbonate (96 mg, 0.694 mmol) were added to the solution of 2-(4-(4-(6-hydroxypyridin-2-yl)piperazin-1-yl)butyl)-4-methyl-1,2,4-triazine-3,5(2*H*,4*H*)dione (**1**) (50 mg, 0.139 mmol) in DMF (volume: 2 mL). The reaction mixture was heated at 80 °C for 4 h. The progress of the reaction was followed by reverse phase HPLC (Phenomenex, Prodigy ODS(3) 4.6 × 250 mm, 5 μ; mobile phase 40: 60 acetonitrile: 25 mM Na_2HPO_4, flow rate 2 mL/min; the precursor appeared at 4.9 min and the fluoride appeared at 7.6 min). After the complete consumption of the phenolic precursor by HPLC, the reaction mixture was cooled to rt, diluted with water, extracted with ethyl acetate (3 × 30 mL). The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated under vacuum. The crude residue was column chromatographed using 2% methanol in CH_2Cl_2 to give the fluoroethyl-CUMI-101 (**2**) as a viscous liquid (37 mg, 65%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 (s, 1H), 6.96 (dq, J = 5.8, 3.2 Hz, 3H), 6.84 (dd, J = 5.8, 3.2 Hz, 1H), 4.87–4.80 (m, 1H), 4.75–4.67 (m,

1H), 4.34–4.26 (m, 1H), 4.25–4.16 (m, 1H), 4.02 (t, J = 7.1 Hz, 2H), 3.34 (s, 3H), 3.13 (br s, 4H), 2.65 (br s, 4H), 2.45 (t, J = 7.6 Hz, 2H), 1.86–1.74 (m, 2H), 1.59 (tt, J = 9.8, 6.1 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.24, 150.98, 148.85, 141.97, 133.77, 122.73, 122.18, 118.47, 113.72, 82.88, 81.19, 67.70, 67.50, 58.08, 53.50, 51.76, 50.46, 26.98, 26.24, 23.72; HPLC (phenomenex, prodigy ODS(3) 4.6 × 250 mm, 5 μ; mobile phase; 40:60 acetonitrile/25 mM Na_2HPO_4, flow rate 2 mL/min, t_R ~7.6 min); HRMS Calcd for $C_{20}H_{29}O_3N_5F$ (MH^+): 406.2254; Found: 406.2268.

4.3. Radiosynthesis of [^{18}F]2-(4-(4-(2-(2-fluoroethoxy)phenyl)piperazin-1-yl)butyl)-4-methyl-1,2,4-triazine-3,5(2*H*,4*H*)-dione([^{18}F]FECUMI-101)

An aqueous solution of [^{18}F]fluoride was treated with 200 μL of 15:1 acetonitrile: water containing kryptofix K_{222} (36 mg) and potassium carbonate (2 mg). The reaction mixture was azeotropically heated and dried at 98° C under a stream of argon by the repeated addition of acetonitrile (3 × 0.5 mL). A solution of 4–5 mg of diethyl tosylate in 1 mL of anhydrous acetonitrile was then added to the reaction vial, sealed, and heated for 10 min at 95 °C. The reaction mixture was allowed to cool to room temperature and then injected onto a semipreparative HPLC column (Phenomenex, Prodigy ODS-Prep 10 × 250 mm, 10 μ; and eluted with 45:55 acetontrile: 1 M ammonium formate. The [^{18}F]fluoroethyltosylate eluted at 7–8 min was collected based on the γ-detector, diluted with 200 mL of deionized water and passed through a Lichrolut EN® column. The column was dried under a stream of argon and eluted with 3 mL of diethyl ether. The ether layer was concentrated under argon without heating and the residue was treated with 2 mg of precursor in 150 μL of DMSO followed by 25 μL of 5% aqueous K_2CO_3 solution (1.6 equiv). The reaction mixture was then heated at 110 °C for 20 min, allowed to cool to rt and injected onto a semi-preparative HPLC column (Prodigy ODS-Prep 10 × 250 mm, 10 μ; mobile phase: 40:60 acetonitrile: 25 mM Na_2HPO_4, flow rate 10 mL/min). The product fraction with a retention time (t_R) of 10–11 min based on γ-detector was collected, diluted with 100 mL of deionized water and subsequently passed through a classic C-18 Sep-Pak cartridge, washed with 10 mL of deionized water and eluted with 1 mL of ethanol. Reconstitution of the product in 1 mL of absolute ethanol afforded [^{18}F]FECUMI-101 in 45 ± 5% yield (EOS) with specific activity in the range 92.5 ± 18.5 GBq/μmol. A portion of the ethanol solution was analyzed by analytical HPLC (Phenomenex, Prodigy ODS(3) 4.6 × 250 mm, 5 μ; mobile phase; 40:60 acetonitrile/25 mM Na_2HPO_4, flow rate 2 mL/min, t_R = 7–8 min) to determine the specific activity, chemical and radiochemical purities. The ethanol solution was then diluted to a volume of 10 mL with saline and filtered through a sterile environment, and a portion of this solution was formulated for injection.

A one-pot modification of the procedure was later developed to provide [^{18}F]FECUMI-101, albeit, in slightly lower yields. Accordingly, a solution of 4–5 mg of diethyl tosylate in 1 mL of anhydrous acetonitrile was added to azeotropically dried [^{18}F]KF/kryptofix and heated for 10 min at 95 °C. The reaction mixture was then allowed to cool to room temperature, diluted with 20 mL of deionized water and passed through a Phenex nylon filter (22 μ) connected to a classic C-18 Sep-Pak® cartridge. The Sep-Pak® was washed with 10 mL of water followed by 1 mL of hexane and dried under a stream of argon for 2 min and eluted with 3 mL of diethyl ether. The ether layer was concentrated under argon without heating and the residue was heated at 110 °C for 20 min with 2 mg of precursor in 150 μL of DMSO and 25 μL of 5% aqueous K_2CO_3 solution (1.6 equiv) and column chromatographed as in the two-step procedure to yield [^{18}F]FECUMI-101 in 20–25% yield (EOS).

4.4. PET studies in baboons

PET studies were performed according to a protocol approved by the Institutional Animal Care and Use Committee of Columbia University Medical Center and New York State Psychiatric Institute. PET scans were performed in two male baboons with an ECAT EXACT HR+ scanner (CPS/Knoxville, TN). The fasted animal was immobilized with ketamine (10 mg/kg, im) and anesthetized with 1.5–2.0% isoflurane via an endotracheal tube. Core temperature was kept constant at 37 °C with a heated water blanket. An intravenous infusion line with 0.9% NaCl was maintained during the experiment and used for hydration and radiotracer injection. An arterial line was placed for obtaining arterial samples for the input function. The head was positioned at centre of the field of view, and a 10 min transmission scan was performed before the tracer injection. For each scan, 111 ± 18 MBq of [^{18}F]FECUMI-101 (specific activity of 93 ± 18 GBq/μmol) was injected as an iv bolus and emission data were collected for 180 min in 3-D mode. Plasma samples were taken every 10 s for the first 2 min, using an automatic system, and thereafter manually for a total of 30 samples over 180 min. Blocking studies were performed by pretreatment with WAY100635 (maleate salt, 0.5 mg/kg/iv) in 12 mL saline, at 50–60 min of the scan. Images were transferred into the image analysis software MEDx (Sensor Systems, Inc., Sterling, Virginia) for drawing and storing regions of interest. All PET images were co-registered within a dynamic study to the previous frame using the Functional Magnetic PET frames are then co-registered to the MRI using FLIRT. Regions of interest drawn on the animal's MRI scan were transferred to co-registered automated image registration (AIR) frames of PET data. Radioactivity levels in the right and left regions were averaged.

4.5. Protein binding and metabolite analyses

The protein binding of [^{18}F]FECUMI-101 in baboon blood samples were determined as described elsewhere.[31,43] Briefly, blood samples were taken at 2, 12, 30, 60, 90, 120, 150 and 180 min after radioactivity injection for metabolite analysis. The supernatant liquid obtained after centrifugation of the blood sample at 2000 rpm for 1 min was transferred (0.5 mL) into a tube and mixed with acetonitrile (0.7 mL). The resulting mixture was vortexed for 10 s, and centrifuged at 14,000 rpm for 4 min. The supernatant liquid (1 mL) was removed and the radioactivity was measured in a well-counter and the majority (0.8 mL) was subsequently injected onto the HPLC column (Phenomenex, Prodigy ODS (3) 4.6 × 250 mm, 5 μ; mobile phase: mobile phase: acetonitrile/25 mM Na_2HPO_4, 40:60 (v/v), flow rate: 2 mL/min, retention time: 7 min) connected to a Waters guard column (Resolve™ 10 μm, 90 A°) equipped with a radioactivity detector. The metabolite and free fractions were collected using a Bioscan gamma detector. All the acquired data were then subjected to correction for background radioactivity and physical decay to calculate the percentage of the parent compound in the plasma at different time points. In order to reaffirm that the retention time of the parent had not shifted during the course of the metabolite analysis, a quality control sample of [^{18}F]FECUMI-101 was injected at the beginning and the end of the study. The percentage of radioactive parent obtained was used for the measurement of metabolite-corrected arterial input functions.

5. Conclusion

The synthesis, radiosynthesis and in vitro pharmacological evaluation of [^{18}F]FECUMI-101, a 5-HT_{1A}R agonist has been achieved. Total time required for the synthesis of [^{18}F]FECUMI-101 is 60 min from EOB using [^{18}F]fluoroethyl tosylate in 45% yield at

EOS with excellent chemical and radiochemical purities and high specific activity. PET studies of [^{18}F]FECUMI-101 in anesthetized baboon showed that the tracer penetrates the BBB and is retained in brain regions corresponding to 5-HT_{1A}R binding, except for thalamus. The specificity of radioligand binding was established by chase studies with WAY100635. Our studies suggests that [^{18}F]FECUMI-101 can be a useful PET tracer for imaging 5-HT_{1A}R in baboon. However, further studies are needed to establish the source of thalamus binding of [^{18}F]FECUMI-101.

Acknowledgments

This work was supported by research Grants from the National Institute of Health (R21 MH077161 and K08 MH76258). The authors thank Dr. Bryan Roth and the NIMH-PDSP program for the competitive receptor-transporter binding and functional assays.

A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2013.05.050.

References and notes

1. Hoyer, D.; Hannon, J. P.; Martin, G. R. *Pharmacol. Biochem. Behav.* **2002**, *71*, 533.
2. Hoyer, D.; Clarke, D. E.; Fozard, J. R.; Hartig, P. R.; Martin, G. R.; Mylecharane, E. J.; Saxena, P. R.; Humphrey, P. P. *Pharmacol. Rev.* **1994**, *46*, 157.
3. Cohn, J. B.; Rickels, K. *Curr Med Res Opin.* **1989**, *11*, 304.
4. Cryan, J. F.; Redmond, A. M.; Kelly, J. P.; Leonard, B. E. *Eur. Neuropsychopharmacol.* **1997**, *7*, 109.
5. Blier, P.; Abbott, F. V. *J. Psychol. Neurosci.* **2001**, *26*, 37.
6. Tomiki, S.; Vera, B.-V.; Jiri, H.; Bettina, B. *Adv. Ther.* **2008**, *25*, 1037.
7. Frederico, F. G.; Helio, Z., Jr. *CNS Agents Med. Chem.* **2010**, *10*, 207.
8. Elena, A.; Rupert, L.; Siegfried, K. *Biol. Psychiatry* **2009**, *66*, 627.
9. Robinson, D. S. *Prim. Psychiatry* **2007**, *14*, 22.
10. Simon, J. S.; Nemeroff, C. B. *J. Clin. Psychiatry* **2005**, *66*, 1216.
11. Moresco, R. M.; Matarrese, M.; Fazio, F. *Curr. Top. Med. Chem.* **2006**, *6*, 2027.
12. Veltman, D. J.; Ruhe, H. G.; Booij, J. *Curr. Pharm. Des.* **2010**, *16*, 1979.
13. Fujita, M. F.; Dennis, S. C.; Innis, R. B. *Biol. Psychiatry* **2000**, *48*, 801.
14. Kumar, J. S. D.; Mann, J. J. *Drug Dev. Today* **2007**, *12*, 748.
15. Passchier, J.; van Waarde, A. *Eur. J. Nucl. Med.* **2001**, *28*, 113.
16. Savitz, J.; Lucki, I.; Drevets, W. C. *Prog. Neurobiol.* **2009**, *88*, 17.
17. Paterson, L. M.; Kornum, B. R.; Nutt, D. J.; Pike, V. W.; Knudsen, G. M. *Med. Res. Rev.* **2013**, *33*, 54.
18. Drevets, W. C.; Thase, M. E.; Moses-Kolko, E. L.; Price, J.; Frank, E.; Kupfer, D. J.; Mathis, C. *Nucl. Med. Biol.* **2007**, *34*, 865.
19. Didelot, A.; Ryvlin, P.; Lothe, A.; Merlet, I.; Hammers, A.; Mauguière, F. *Brain* **2008**, *131*, 2751.
20. Aznavour, N.; Zimmer, Luc *Neuropharmacology* **2007**, *52*, 695.
21. Sullivan, G. M.; Ogdan, T.; Ocqendo, M.; Kumar, J. S. D.; Simpson, N.; Huang, Y.; Mann, J. J.; Parsey, R. V. *Biol. Psychiatry* **2009**, *66*, 223.
22. Gozlan, H.; Thibault, S.; Laporte, A. M.; Lima, L.; Hamon, M. *Eur. J. Pharmacol.* **1995**, *288*, 173.
23. Burnet, P. W.; Eastwood, S. L.; Harrison, P. J. *Neurochem. Int.* **1997**, *30*, 565.
24. Bantick, R. A.; Rabiner, E. A.; Hirani, E.; de Vries, M. H.; Hume, S. P.; Grasby, P. M. *Neuropsychopharmacology* **2004**, *29*, 847.
25. Laruelle, M. *J. Cereb. Blood Flow Metab.* **2000**, *20*, 423.
26. Rabiner, E. A.; Messa, C.; Sargent, P. A.; Husted-Kjaer, K.; Montgomery, A.; Lawrence, A. D.; Bench, C. J.; Gunn, R. N.; Cowen, P.; Grasby, P. M. *Neuroimage* **2002**, *15*, 620.
27. Udo, D. H. J. I.; Bosker, F. J.; Van Waarde, A.; Pruim, J.; Willemsen, A. T. M.; Vaalburg, W.; Den, B. J. A. *Synapse* **2002**, *46*, 108.
28. Rabiner, E. A.; Gunn, R. N.; Wilkins, M. R.; Sargent, P. A.; Mocaer, E.; Sedman, E.; Cowen, P. J.; Grasby, P. M. *Nucl. Med. Biol.* **2000**, *27*, 509.
29. Passchier, J.; van Waarde, A.; Pieterman, R. M.; Willemsen, A. T.; Vaalburg, W. *Psychopharmacology* **2001**, *155*, 193.
30. Riad, M.; Zimmer, L.; Rbah, L.; Watkins, K. C.; Hamon, M.; Descarries, L. *J. Neurosci.* **2004**, *24*, 5420.
31. Arlicot, N.; Vercouillie, J.; Ribeiro, M.-J.; Tauber, C.; Venel, Y.; Baulieu, J.-L.; Maia, S.; Corcia, P.; Stabin, M. G.; Reynolds, A.; Kassiou, M.; Guilloteau, D. *Nucl. Med. Biol.* **2012**, *39*, 570.
32. Ettrup, A.; Hansen, M.; Santini, M.; Paine, J.; Gillings, N.; Palner, M.; Lehel, S.; Herth, M.; Madsen, J.; Kristensen, J.; Begtrup, M.; Knudsen, G. *Eur. J. Nucl. Med. Mol. Imaging* **2011**, *38*, 681.
33. Narendran, R.; Martinez, D.; Mason, N. S.; Lopresti, B. J.; Himes, M. L.; Chen, C.-M.; May, M. A.; Price, J. C.; Mathis, C. A.; Frankle, W. G. *Synapse* **2011**, *65*, 1344.
34. Shotbolt, P.; Tziortzi, A. C.; Searle, G. E.; Colasanti, A.; van der Aart, J.; Abanades, S.; Plisson, C.; Miller, S. R.; Huiban, M.; Beaver, J. D.; Gunn, R. N.; Laruelle, M.; Rabiner, E. A. *J. Cereb. Blood Flow Metab.* **2012**, *32*, 127.
35. Tomasi, G.; Nabulsi, N.; Zheng, M.-Q.; Weinzimmer, D.; Ropchan, J.; Blumberg, L.; Brown-Proctor, C.; Ding, Y.-S.; Carson, R. E.; Huang, Y. *J. Nucl. Med.* **2013**, *54*, 600.
36. Kumar, J. S. D.; Prabhakaran, J.; Majo, V. J.; Milak, M. S.; Hsiung, S.-C.; Tamir, H.; Simpson, N. R.; Van Heertum, R. L.; Mann, J. J.; Parsey, R. V. *Eur. J. Nuc. Med. Mol. Imaging* **2007**, *34*, 1050.
37. Milak, M. S.; Mann, J. J.; Ogden, T. R.; Kumar, J. S. D.; Prabhakaran, P.; Majo, V. J.; Pratap, M.; Parsey, R. V. *J. Nucl. Med.* **2008**, *49*, 587.
38. Milak, M. S.; Severance, A. J.; Ogden, T. R.; Prabhakaran, J.; Kumar, J. S. D.; Majo, V. J.; Mann, J. J.; Parsey, R. V. *J. Nucl. Med* **2010**, *51*, 1892.
39. Kumar, J. S. D.; Bai, B.; Ng, H. H.; Mirsalis, J. C.; Eralandsson, K.; Milak, M. S.; Majo, V. J.; Prabhakaran, J.; Mann, J. J.; Parsey, R. V. *Int. J. Toxicol.* **2011**, *30*, 611.
40. Milak, M. S.; Severance, A. J.; Ogden, T. R.; Prabhakaran, J.; Kumar, J. S. D.; Majo, V. J.; Mann, J. J.; Parsey, R. V. *JCBFM* **2011**, *31*, 241.
41. Palner, M.; Underwood, M. D.; Kumar, J. S. D.; Arango, V.; Knudsen, G. M.; Mann, J. J.; Parsey, R. V. *Synapse* **2011**, *65*, 715.
42. Kumar, J. S. D.; Majo, V. J.; Milak, M. S.; Prabhakaran, J.; Mali, P.; Savenkova, L.; Mann, J. J.; Parsey, R. V. *J. Pharm. Sci.* **2012**, *120*, 254.
43. Bauman, A.; Piel, M.; Schirrmacher, R.; Rosch, F. *Tetrahedron Lett.* **2003**, *44*, 9165.
44. Wilson, A. A.; Jin, L.; Garcia, A.; DaSilva, J. N.; Houle, S. *Appl. Radiat. Isot.* **2001**, *54*, 203.
45. *Fluorine and Health*; Tressaud, A., Haufe, G., Eds.; Elsevier, 2008.
46. Champion, C.; Le Loirec, C. *Phys. Med. Biol.* **2007**, *52*, 6605.
47. Sorensen, J. A.; Phelps, M. E. *Physics in Nuclear Medicine*; W B Sanders: Philadelphia, 1987.
48. Kumar, J. S. D.; Majo, V. J.; Tamir, H.; Millak, M. S.; Hsing, S.-C.; Prabhakaran, J.; Simpson, N. R.; Van Heertum, R. L.; Mann, J. J.; Parsey, R. V. *J. Med. Chem.* **2006**, *49*, 125.
49. Herth, M. M.; Volk, B.; Pallagi, K.; Kofoed Bech, L.; Antoni, F. A.; Knudsen, G. M.; Kristensen, J. L. *ACS Chem. Neurosci.* **2012**, *3*, 1002.
50. Missale, C.; Nash, R. S.; Robinson, S. W.; Jaber, M.; Caron, C. G. *Physiol. Rev.* **1998**, *78*, 189.
51. Patel, N. H.; Vyas, N. R.; Puri, B. K.; Nijran, K. S.; Al-Nahhas, A. *J. Nucl. Med.* **2010**, *51*, 511.
52. Jones, L. S.; Miller, G.; Gauger, L. L.; Davis, J. N. *Life Sci.* **1985**, *36*, 45.
53. Gross-Isseroff, R.; Dillon, K. A.; Fieldust, S. J.; Biegon, A. *Arch. Gen. Psychiatry* **1990**, *47*, 1049.
54. Szot, P.; White, S. S.; Greenup, J. L.; Leverenz, J. B.; Peskind, E. R.; Raskind, M. A. *Mol. Brain Res.* **2005**, *139*, 367.
55. Chemel, B.; Roth, B.; Armbruster, B.; Watts, V.; Nichols, D. *Psychopharmacology* **2006**, *188*, 244.