Synthesis and in Vivo Validation of [*O*-Methyl-^{11}C]2-{4-[4-(7-methoxynaphthalen-1-yl)piperazin-1-yl]butyl}-4-methyl-2*H*-[1,2,4]triazine-3,5-dione: A Novel 5-HT$_{1A}$ Receptor Agonist Positron Emission Tomography Ligand

J. S. Dileep Kumar,*,† Vattoly J. Majo,† Shu-Chi Hsiung,§ Matthew S. Millak,†,§ Kuo-Peing Liu,§ Hadassah Tamir,†,§ Jaya Prabhakaran,† Norman R. Simpson,‡,§ Ronald L. Van Heertum,‡,§ J. John Mann,†,‡,§ and Ramin V. Parsey†,§

Departments of Psychiatry and Radiology, Columbia University College of Physicians and Surgeons and Department of Neuroscience, New York State Psychiatric Institute, New York, New York 10032

Received July 27, 2005

Antagonist 5-HT$_{1A}$ PET ligands are available, but an agonist ligand would give more information about signal transduction capacity. Synthesis and in vivo evaluation of [*O*-methyl-^{11}C]2-{4-[4-(7-methoxynaphthalen-1-yl)piperazin-1-yl]butyl}-4-methyl-2*H*-[1,2,4]triazine-3,5-dione (**10**), a potential high affinity (K_i = 1.36 nM) 5-HT$_{1A}$ agonist PET tracer is described. Piperazine **10** is a 5-HT$_{1A}$ agonist with an EC_{50} comparable to serotonin, based on cAMP formation and GTP$_\gamma$S binding assays. Radiosynthesis of [^{11}C]**10** has been achieved by reacting 2-{4-[4-(7-hydroxynaphthalen-1-yl)piperazin-1-yl]butyl}-4-methyl-2*H*-[1,2,4]triazine-3,5-dione (**9**) and [^{11}C]CH_3OTf in 25 ± 5% (n = 15) yield at the end of synthesis (EOS). The chemical and radiochemical purities of [^{11}C]**10** were >99% with a specific activity 1500 ± 300 Ci/mmol (n =15). PET studies in anesthetized baboon demonstrate [^{11}C]**10** specific binding in brain regions rich in 5-HT$_{1A}$ receptors. Binding of [^{11}C]**10** was blocked by WAY100635 and 8-OH-DPAT. The regional brain volumes of distribution (V_T) of [^{11}C]**10** in baboon correlate with [^{11}C]WAY100635 V_T in baboons. These data provide evidence that [^{11}C]**10** is the first promising agonist PET tracer for the 5-HT$_{1A}$ receptors.

Introduction

The 5-HT$_{1A}$ serotonin receptor is implicated in the pathophysiology of major neuropsychiatric disorders including depression, suicidal behavior, panic disorder, epilepsy, bulimia, schizophrenia, Parkinson's disease and Alzheimer's disease and is therefore an important target for drug therapy.[1−8] 5-HT$_{1A}$ receptors are expressed as somatodendritic autoreceptors in serotonin neurons of the raphe nuclei (presynaptic) and as postsynaptic receptors in cortical and subcortical brain regions.[9,10] Postsynaptic 5-HT$_{1A}$ receptors are found in limbic regions (hippocampus, septum) > prefrontal and entorhinal cortices > amygdala > striatum and the lowest in cerebellum.[11,12] Positron emission tomography (PET) is the best method to measure 5-HT$_{1A}$ receptor binding in living human brain, and the antagonist radiotracer [^{11}C]WAY-100635 is the most commonly used 5-HT$_{1A}$ ligand for in vivo studies.[13] However, the 5-HT$_{1A}$ receptor exists in high and low agonist affinity states,[14,15] and antagonist ligands bind both the high affinity (HA) and low affinity (LA) conformations of the 5-HT$_{1A}$ receptor with comparable affinity. In contrast, agonists bind preferentially to the HA state of the receptor which is coupled to G-protein, and therefore agonist binding provides a more meaningful functional measure of the 5-HT$_{1A}$ receptor.

Development of an agonist radioligand would provide several potential advantages over antagonist radioligands. First, it may enable the determination of the HA:LA ratio in vivo in human brain. Agonists, some used as therapeutic agents, such as flesinoxan, buspirone and gepirone bind preferentially to HA sites of 5-HT$_{1A}$ receptor.[16,17] In vitro, the ratio of [^{3}H]8-OH-DPAT:[^{3}H]WAY-100635 (HA:LA + HA) binding in human neocortex is 1.7−2.7;[18] the in vivo ratio is *not known*. Second, it has been suggested that agonist ligands may be more sensitive to intrasynaptic levels of endogenous neurotransmitter such as serotonin or dopamine.[19] Antagonist radiotracers bind to both the HA and LA receptors. As serotonin is an agonist which binds preferentially to the HA state of the receptor, serotonin may only measurablely displace antagonist radiotracer binding to the HA receptors. In contrast, all agonist radiotracer binding could be displaced by increases in synaptic serotonin. As an example, D_2 receptor agonist radioligands are more sensitive to displacement by dopamine than antagonist radioligands.[20−22] Finally, an agonist radioligand would provide a better estimate of receptor occupancy for agonist therapeutic agents such as gepirone because of a better signal-to-noise ratio. 5-HT$_{1A}$ receptor agonism has been postulated to play a role in the action of atypical antipsychotic drugs (APDs) that achieve an antipsychotic effect with lower rates of extrapyramidal side effects (EPS) compared to first-generation APDs such as haloperidol.[23]

Several radioligands for 5-HT$_{1A}$ receptor have been evaluated for imaging purposes by PET or SPECT.[24] The most successful radioligands are 5-HT$_{1A}$ receptor antagonists such as [*carbonyl*-^{11}C]WAY-100635 (WAY), [*carbonyl*-^{11}C]desmethyl-WAY-100635 (DWAY), and *p*-[^{18}F]MPPF.[25−27] Considering the merits of 5-HT$_{1A}$ receptor agonist tracers, several efforts are being made for the development of imaging agents. Most imaging studies have been carried out with the aminotetralin structural skeleton (Chart 1). A number of radioiodinated derivatives such as [^{123}I]-BH-8-MeO-*N*PAT and [^{123}I]-8-OH-PIPAT have been synthesized and evaluated in vitro.[28−30] However, in vivo data with these radioligands have not been reported probably due to the relatively high lipophilicity or high protein binding. The orally active 5-HT$_{1A}$ receptor agonist OSU 191 (IC_{50} = 1.2 nM) has been [^{11}C]-labeled, but no PET studies were reported.[31] We

* Corresponding author. Telephone: (212) 543-5909. Fax: (212) 543-1054. E-mail: dkumar@neuron.cpmc.columbia.edu.

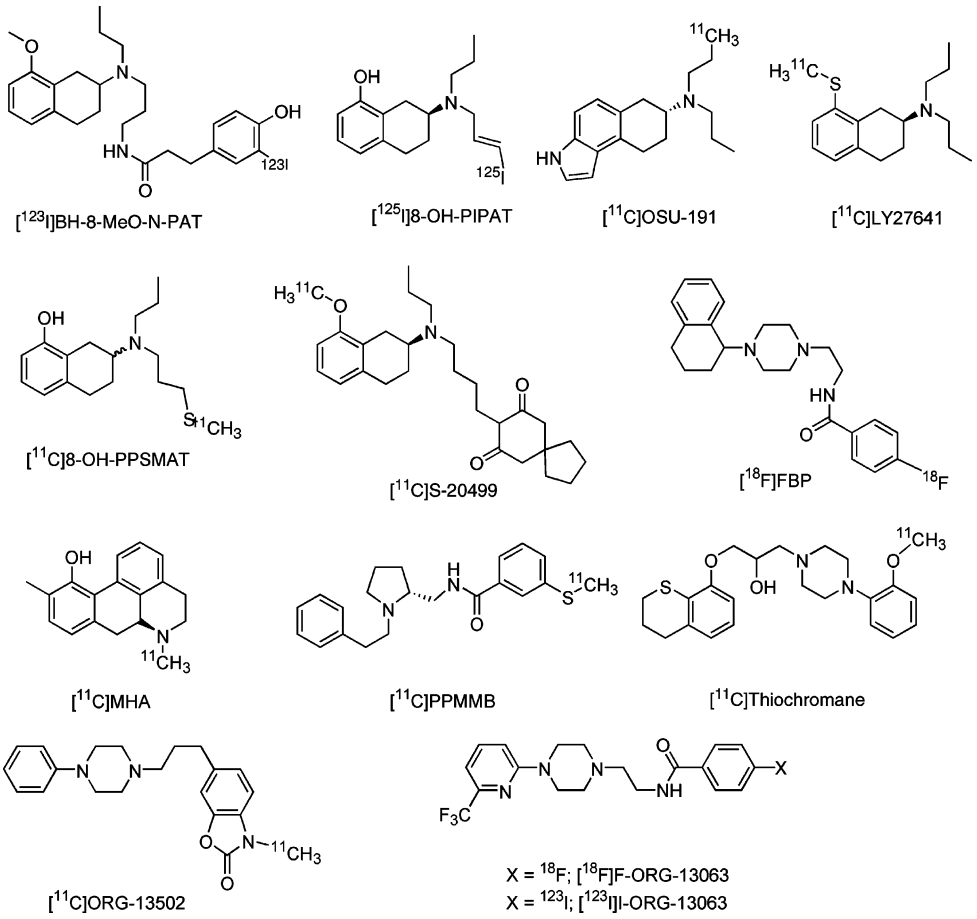
† Department of Psychiatry, Columbia University College of Physicians and Surgeons.

‡ Department of Radiology, Columbia University College of Physicians and Surgeons.

§ Department of Neuroscience, New York State Psychiatric Institute.

10.1021/jm050725j CCC: $33.50
Published on Web 12/02/2005

Chart 1



have previously labeled LY-274601 (K_i 0.6 nM), a full agonist at the 5-HT_{1A} receptor, but in vivo it lacked detectable specific binding and was very rapidly metabolized in mice.[32] Similarly, [^{11}C]-labeled (±)-8-OH-PPSMAT and (+)S-20499 also did not show specific binding in rats.[33] Experiments in monkeys with the [^{11}C]-analogue of *(R)*-11-hydroxy-10-methylaporphine, a potent and selective 5-HT_{1A} receptor agonist (K_i = 0.45 nM), were also unsuccessful.[34] A number of agonist radioligands, which possess favorable pharmacological characteristics, such as [^{11}C]ORG-13502, [^{11}C](S)-PPMMB, [^{18}F]FBP, [^{18}F] and [^{123}I] analogues of ORG 13063, and [^{11}C]-analogue of a thiochroman derivative, failed to demonstrate specific binding in vivo.[35−40] Thus, although the antagonist tracers, [^{11}C]WAY and *p*-[^{18}F]MPPF, are established PET ligands in clinical studies, a successful 5-HT_{1A} receptor agonist PET radiotracer for studies in living brain has not yet been developed.

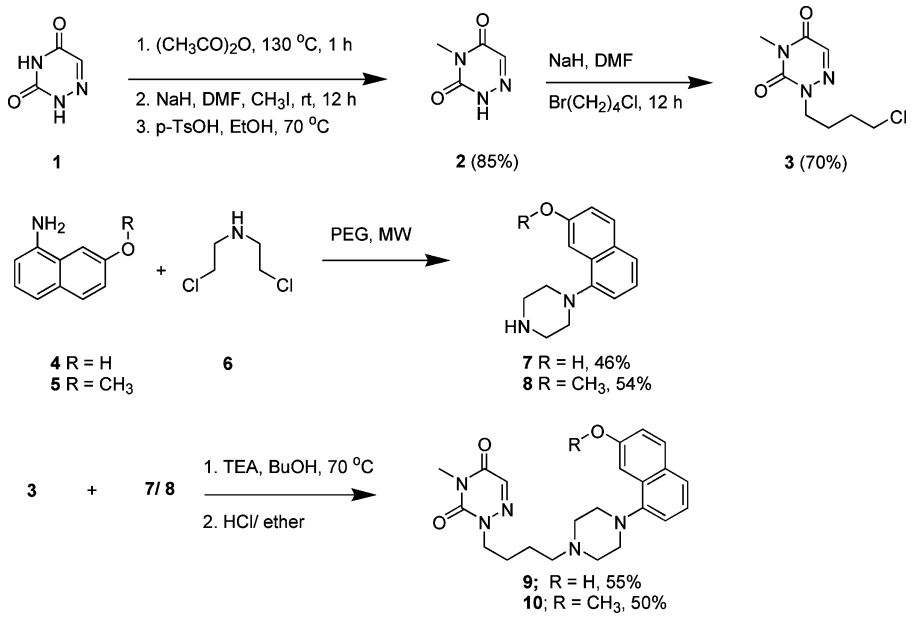
Successful agonist radiotracers have been recently developed for D_2 and M_2 receptors.[22,41] We sought therefore to develop an agonist PET probe to image $5HT_{1A}$ and selected 2-{4-[4-(7-methoxynaphthalen-1-yl)piperazin-1-yl]-butyl}-4-methyl-2*H*-[1,2,4]triazine-3,5-dione (**10** or MPT or F11461) as our candidate ligand due to its high affinity to 5-HT_{1A} receptor (pK_i 10.49)[42,43] and favorable lipophilicity (calculated octanol−water partition coefficient (clog*P*) = 1.8, calculated with ACD/log*P* DB program).

Results and Discussion

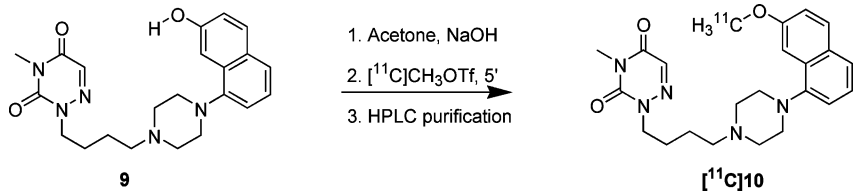
Chemistry. The synthesis of **10** was established from commercial 6-azauracil in five steps (Scheme 1). Accordingly, 6-azauracil (**1**) was selectively acetylated at the 2-position by refluxing in acetic anhydride. Methylation of the resulting product at the 4-position by sodium hydride in DMF and methyl iodide followed by treatment with catalytic *p*-TsOH in refluxing ethanol yielded **2** in 76% overall yield. The tethering of the 4-chlorobutyl side chain in compound **2** was achieved by alkylation with 1-bromo-4-chlorobutane in the presence of sodium hydride in DMF to afford 2-(4-chlorobutyl)-4-methyl-2*H*-[1,2,4]triazine-3,5-dione (**3**) in 70% yield. The required counterpart of compound **3** for the synthesis of the radiolabeling precursor **9**, 8-piperazin-1-ylnaphthalen-2-ol (**7**), was synthesized by the reaction of 8-aminonaphthalen-2-ol (**4**) and bis-(2-chloroethyl)amine **(6)** in poly(ethylene glycol) under microwave conditions with 46% yield. Under identical conditions 1-(7-methoxynaphthalen-1-yl)piperazine (**8**), the precursor for the synthesis of **10** was obtained in 54% yield by coupling 7-methoxynaphthalen-1-ylamine (**5**) and compound **6**. Coupling reactions under thermal conditions resulted in an inseparable mixture of products. The synthesis of **9** was achieved by coupling compound **3** with naphthylamine **7** in 55% yield. Under similar conditions, coupling of **4** with naphthylamine **8** provided piperazine **10** in 50% yield (Scheme 1).

Radiochemistry. The labeling of the desmethyl compound **9** was initially modeled with nonradioactive methyl triflate and methyl iodide, and the reaction conditions were optimized using NaOH as base. The optimal condition for radiosynthesis of [^{11}C]**10** was eventually identified as treating the radiolabeling precursor **9** with [^{11}C]CH_3OTf in acetone in the presence of 10 μL of 5 M NaOH at room temperature (Scheme 2, Figure 1). The product was purified by RP-HPLC followed by passing through a C-18 Sep-Pak cartridge. The radiochemical yield of [^{11}C]**10** was 25 ± 5% (n = 15) at the end of synthesis (EOS). The chemical identity of [^{11}C]**10** was established by coinjecting

Scheme 1



Scheme 2



the radioactive product with an authentic sample of nonradioactive standard **10** on an analytical RP-HPLC. The chemical and radiochemical purities of [^{11}C]**10** were found to be >99% with a specific activity 1500 ± 300 Ci/mmol (n = 10) at end of bombardment (EOB). The average time required for the [^{11}C] labeling was 30 min (EOB). The partition coefficient (logP_{oct}) of [^{11}C]**10** was determined by standard shake flask method[44] and found to be 2.2.

In Vitro Sensitivity and Selectivity. Competition binding studies with [^{3}H]8-OH-DPAT at various concentrations determined the inhibitory constant values (K_i) of **10** and 5-HT to be 1.36 nM and 1.58 nM, respectively (Figure 2). The affinity of **10** for various brain receptors and transporters was assessed through the National Institute of Mental Health-Psychoactive Drug Screening Program (NIMH-PDSP), and results are summarized in Table 1. Although the assay revealed nanomolar affinity of compound **10** to 5-HT$_7$ (K_i = 9.1 nM) and D$_4$ (K_i = 8.5 nM) receptors, the number of these receptors is too small in brain to detect using PET.[45−47] The next lowest K_is of **10** for other tested targets were about 20 times more than 5-HT$_{1A}$ receptor [5-HT$_{2A}$ (K_i = 41.5), D$_2$ (K_i = 22.1 nM), α_1 (K_i = 32.2 nM), α_{2c} (K_i = 23.7 nM), β_2 receptors (K_i = 60.6 nM)]. The affinity for other brain receptors and transporters was low (0.1 to 10 μM).

In Vitro Agonist Activity of 10. Agonism of **10** at 5-HT$_{1A}$ receptor was assessed using guanosine 5′-*O*-(3-[^{35}S]thiotriphosphate) ([^{35}S]GTPγS) binding and cyclic adenosine monophosphate (cAMP) formation in membranes of Chinese hamster

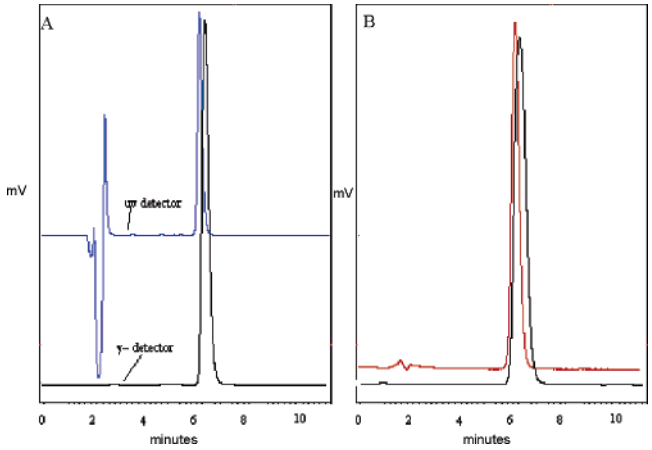


Figure 1. A. Radiosynthesis of [^{11}C]-**10**. B. Coinjection with nonradioactive **10**.

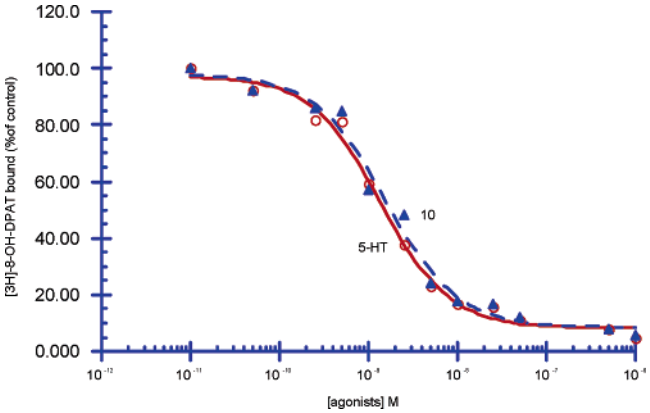


Figure 2. Competition of [^{3}H]-8-OH-DPAT binding by 5-HT and **10** in CHO membranes. Values are shown expressed as a percentage of control which is the binding of [^{3}H]-8-OH-DPAT in the absence of **10** or 5-HT. Data points are means of duplicate determinations from representative experiments repeated on at least three independent occasions with similar results.

Table 1. K_is of **10** for Receptors and Transporters

targets	K_i (nM)	selectivity[a]	targets	K_i (nM)	selectivity
5-HT_{1A}	1. 36 ± 0.16	1	D_1	7396	5438
5-HT_{2A}	41.5 ± 4.3	30	D_2	22.01 ± 1.3	16.25
5-HT_{2C}	99.6 ± 16.8	73	D_3	62.8 ± 21.66	46
5-HT_3	>10000	>10000	D_4	8.5 ± 1.25	6.2
5-HT_{5a}	228.6 ± 56	167	D_5	>10000	>10000
5-HT_6	120.3 ± 30	88	DAT	407.4 ± 94	300
5-HT_7	9.1 ± 0.76	6.7	DOR	>10000	>10000
A_2, A_3, A_4	>10000	>10000	EP	>10000	>10000
adrenergicα_1	32.2 ± 1.3	23.6	GABA	>10000	>10000
adrenergicα_{2A}	1012	744	H_1	150 ± 39	111
adrenergicα_{2B}	316.2 ± 37	232	H_2	302 ± 48	222
adrenergic$_{2C}$	23.7 ± 9.64	17.4	H_3, H_4	>10000	>10000
adrenergicβ_1	475.8 ± 78	350	hERG	>10000	>10000
adrenergicβ_2	60.6 ± 10.8	44.5	KOR	>10000	>10000
adrenergicβ_3	>10000	>10000	M	>10000	>10000
BZP	>10000	>10000	MDR1	>10000	>10000
Ca^+ channel	>10000	>10000	MOR	>10000	>10000
AMPA	>10000	>10000	mGluR	>10000	>10000
NET	6980	5132	NMDA	>10000	>10000
NK	>10000	>10000	SERT	6144	4517
$Sigma_1$	480 ± 173	352	$Sigma_2$	>10000	>10000
V_1, V_2	>10000	>10000	$VMAT_{1,2}$	>10000	>10000
Na^+ channel	>10000	>10000	NT_1	>10000	>10000
CB_1, CB_2	>10000	>10000	imidazoline	>10000	>10000

[a] Ratio of the inhibitory constants with respect to 5-HT_{1A} receptor; A: adenosine; α: alpha; β: beta; BZP: benzodiazepine; AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; V: vasopressin; CB: cannabinoid; D: dopamine; DAT: dopamine transporters; DOR: delta opioid receptors; EP: prostanoid receptors: GABA: gamma-amino butyric acid; H: histamine; hERG: human ether-a-go-go; KOR: kappa opioid receptors; M: muscarinic; MDR: multidrug resistance; MOR: mu opioid receptor; mGluR: metabotropic glutamate receptors; NMDA: *N*-methyl-D-aspartic acid; NK: neurokinin; SERT: serotonin transporter; VMAT: vesicular monoamine transporter; NET: norepinephrine transporter; NT: neurotrophin.

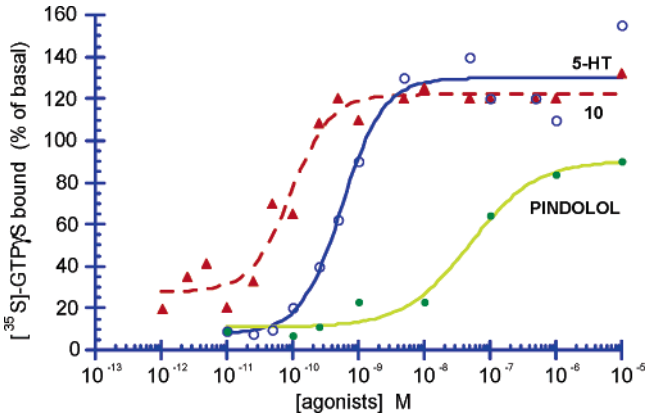


Figure 3. Effect of 5-HT_{1A} receptor agonists concentration on the stimulation of [^{35}S]GTPγS binding in CHO cells. Values are shown as expressed as a percentage above basal which is the binding of [^{35}S]GTPγS in the absence of agonists. Data points are means of duplicate determinations from representative experiments repeated on at least three independent occasions with similar results.

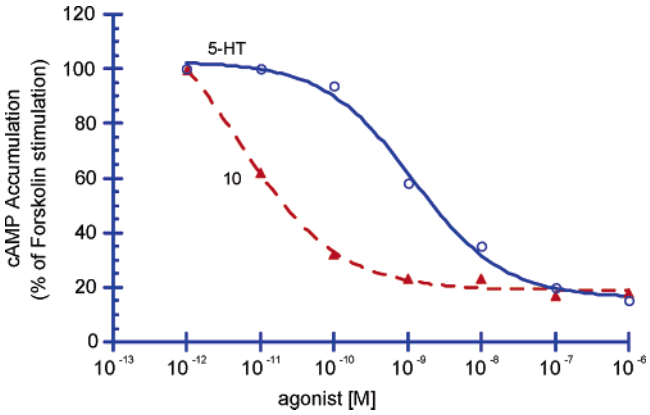


Figure 4. Differential inhibition of forskolin stimulated cAMP accumulation by 5-HT and **10** in CHO membranes. Values are shown expressed as a percentage of forskolin stimulation which is as 100%. Data points are means of duplicate determinations from representative experiments repeated on at least three independent occasions with similar results.

ovary (CHO) cells expressing the receptor. Figure 3 shows the dose−response curves for [^{35}S]GTPγS binding in response to **10**, 5-HT, and pindolol and demonstrates increased binding of [^{35}S]GTPγS over the basal level in a concentration-dependent manner. These experiments were performed in the presence of 3 μM GDP to maximize agonist stimulation of [^{35}S]GTPγS binding relative to the basal signal. At nanomolar concentrations, **10** show saturation of the increase in binding of GTPγS, whereas only 70% and 10% of saturation was observed for 5-HT and pindolol, respectively. The EC_{50} of **10** is about one tenth that of 5-HT (EC_{50} = 0.05 nM and 0.5 nM, respectively). The EC_{50} observed for pindolol, a known 5-HT_{1A} partial agonist, is several log orders higher (Figure 3).

To further evaluate the agonist property of **10**, we determined inhibition of forskolin-stimulated cAMP accumulation by **10** and 5-HT via activation of the 5-HT_{1A} receptor which is coupled to $G_{\alpha i}$ (Figure 4). At one nanomolar concentration, **10** shows a maximal inhibition of cAMP, whereas, with the same concentration, 5-HT expressed approximately 40% inhibition. The EC_{50} of **10** and 5-HT using this method are 0.01 nM and 1 nM, respectively, comparable to the values from measurement by GTPγS binding. The effect of **10** in these two assays shows that **10** is a 5-HT_{1A} agonist with affinity higher than that of endogenous ligand 5-HT.

In Vivo Evaluation of [^{11}C]10 in Baboons. Metabolite-corrected arterial input functions were used to determine the volume of distribution (V_T) of [^{11}C]**10**. The plasma samples at time points 2, 4, 12, 30, 60 and 90 min were extracted with acetonitrile and were used for metabolite assays. Only polar metabolites were observed in the assays and the percentage of unmetabolized [^{11}C]**10** (mean ± SD, n=15) was 90± 2% of total plasma radioactivity at 2 min, 87 ± 3% at 4 min, 65 ± 5% at 12 min, 30 ± 5% at 30 min, 20 ± 3% at 60 min and 12 ± 3% at 90 min respectively (Figure 5). We conducted blockade

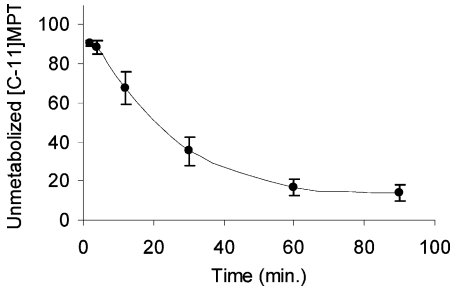


Figure 5. Unmetabolized parent fraction of [^{11}C]**10** in baboon plasma. Filled circles represent the mean fraction in 15 determinations. Error bars are standard deviations.

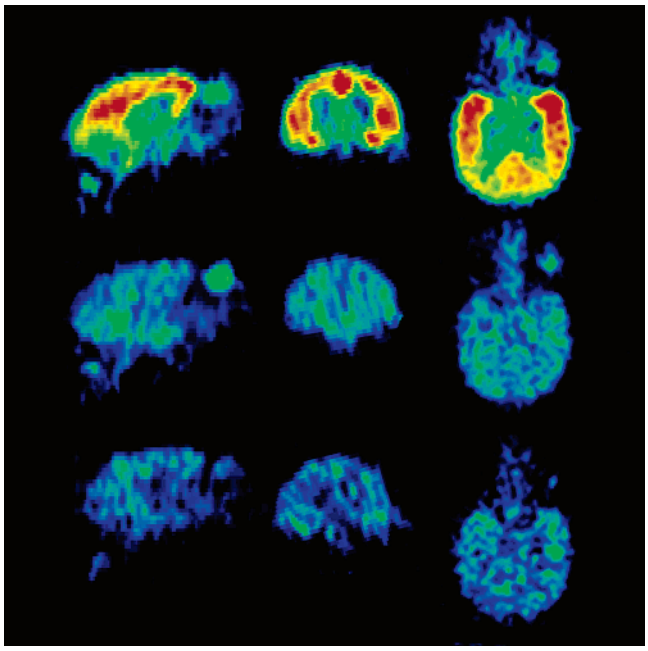


Figure 6. Sum of last 60 min [^{11}C]**10** PET scans in baboon brain (images are normalized to the injected dose). Top row: baseline; middle row: pretreatment with WAY100635 (0.5 mg/kg iv); bottom row: pretreatment with 8-OH-DPAT(2 mg/kg iv). First column: sagittal, middle column: coronal, last column: axial views.

studies in the same baboon and no significant changes in metabolites or free fractions were observed.

[^{11}C]**10** penetrated the BBB and accumulated in brain regions known to possess high concentrations of 5-HT$_{1A}$ receptor (Figure 6, top row). Time activity curves (TACs) showed rapid passage of the tracer across the BBB (Figure 7A). A high correlation (R = 0.885, p = 0.003) between [^{11}C]WAY100635 V_T and [^{11}C]**10** V_T was observed (Figure 7B). The V_T of [^{11}C]WAY100635 was higher compared with [^{11}C]**10**, presumably because [^{11}C]WAY100635, an antagonist, binds to both HA and LA states of 5-HT$_{1A}$ receptor. Cerebellum had the lowest binding of [^{11}C]**10** and [^{11}C]WAY100635, consistent with our previous post-mortem binding study.[1, 48]

We examined the in vivo specificity of [^{11}C]**10** by conducting block studies with WAY100635 (0.5 mg/kg iv, 20 min prior to [^{11}C]**10** administration; Figure 6 middle row and Figure 8A) and the 5-HT$_{1A}$ agonist 8-OH-DPAT (2 mg/kg iv, 30 min prior to the administration of [^{11}C]**10** (Figure 6 bottom row and Figure 8B). The TACs show that uptake of [^{11}C]**10** to brain regions with specific binding was reduced to levels found in the cerebellar reference region, demonstrating the in vivo specificity of [^{11}C]**10** binding for 5-HT$_{1A}$ receptor. To determine the degree of in vivo binding of **10** to 5-HT$_{2A}$, D$_2$, D$_3$ and D$_4$ receptors, we conducted block studies with M100907 (5-HT$_{2A}$ antagonist, 2 mg/kg iv, Figure 9A.) and haloperidol (D$_2$, D$_3$ and D$_4$ antagonist, 0.25 mg/kg iv, Figure 9B.) 30 min prior to the administration of [^{11}C]**10**. To evaluate the effect of haloperidol and M100907 on [^{11}C]**10** binding, the specific to nonspecific equilibrium partition coefficient (BP$_2$) was analyzed using a mixed-effects model with M100907 or haloperidol vs baseline status and ROI as fixed effects and individual PET scans and experimental order as random effects. To correct for inhomogeneity of variance, the analysis was performed on log-transformed BP$_2$ data. The model was fit using SPSS software (Chicago, IL) running on Mac OSX (Apple Computer, Inc., Cupertino, California). All p values reported correspond to two-sided alternative hypotheses. Model adequacy of the transformed variables was checked via standard residual-based diagnostics. Neither M100907 nor haloperidol affected V_T of the reference region (F = 7.23, df = 1, p = 0.23; F = 0.67, df=1, p = 0.564 respectively). Binding potential (BP$_2$) differs between brain regions (F = 606.67, df = 4, p < 0.0001). There is no significant M100907 or haloperidol effect on **10** binding in the regions of interest (F = 31.81, df=1, p M100907 0.112; F = 1.63, df=1, p = 0.423 respectively), demonstrating that blocking 5-HT$_{2A}$ or dopamine receptors does not alter **10** binding (BP$_2$). This suggests that **10** does not bind significantly to 5-HT$_{2A}$ or dopamine receptors in vivo at tracer doses.

Experimental Section

The commercial chemicals used in the synthesis were purchased from Sigma-Aldrich Chemical Co., Fisher Scientific Inc. or Lancaster and were used without further purification. Melting points were determined on a Fisher Scientific Melting point apparatus. ^{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 (δ) are reported in parts per million (ppm) relative to tetramethylsilane (TMS). The mass spectra were recorded on JKS-HX 11UHF/HX110 HF Tandem Mass Spectrometer in the fast atom bombardment (FAB+) mode. Microwave experiments were conducted on Sharp carousel model instrument. Elemental analyses of selected compounds as HCl salts were performed by Galbraith Inc. Thin-layer chromatography (TLC) were performed using silica gel 60 F254 plates from Emerck. Semipreparative HPLC analyses were performed using a Waters 1525 HPLC system, Phenomenex, Prodigy ODS(3) 10 × 250 mm, 10 μ column using 40:60 (acetonitrile: 0.1 M ammonium formate solution), 10 mL per minutes flow rates and for analytical studies Phenomenex, Prodigy ODS(3) 4.6 × 250 mm, 5 μ column using 40:60 (acetonitrile: 0.1 M ammonium formate solution), 2 mL per minute as flow rate were used. Flash column chromatography was performed on silica gel (Fisher 200−400 mesh) using the solvent system indicated in the experimental procedure for each compound. [^{11}C]Methyl triflate was synthesized in the Radioligand Laboratory of Columbia University by transferring [^{11}C]methyl iodide through a glass column containing silver triflate (AgOTf) at 200 °C.[49] The radiochemical and chemical purities were analyzed by RP-HPLC with PDA and NaI detectors. Partition coefficient determination was performed with a Packard Instruments Gamma Counter (Model E5005). Metabolite analyses were performed using Waters bondpack C18 column (7.8 × 300 mm) using a mobile phase 40:60 (acetonitrile: 1% phosphoric acid solution). The free fractions and metabolites were measured using Packard Instruments Gamma Counter (Model E5005).

Chemistry. 4-Methyl-2*H*-[1,2,4]triazine-3,5-dione (2). A solution of 6-azauracil (**1**, 2.0 g, 17.70 mmol) in acetic anhydride (10 mL) was refluxed for 2h. The reaction mixture was then cooled to room temperature and dried under vacuum. The crude product was repeatedly treated with toluene and concentrated under vacuum to give the acetyl derivative (1.92 g, 80%). The resultant acetyl derivative (1.92 g, 12.4 mmol) was added to a suspension of NaH

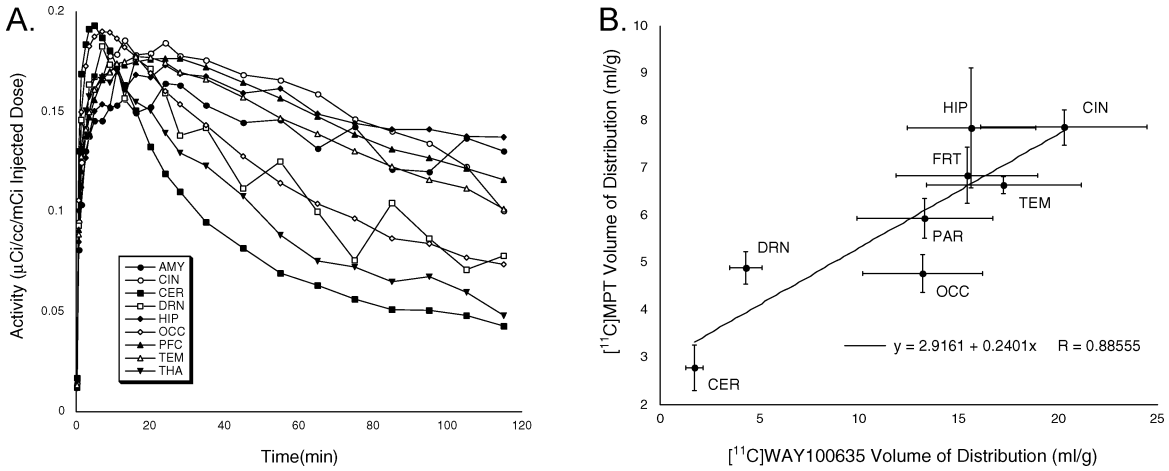


Figure 7. A. Time activity curves of the radioactivity in baboon after the injection of [^{11}C]**10**. B. Correlation between [^{11}C]**10** and [^{11}C]WAY100635 binding in baboons. (AMY = amygdala, CIN = cingulate, CER = cerebellum, DRN = dorsal raphe nucleus, HIP = hippocampus, OCC = occipital cortex, PFC = prefrontal cortex, TEM = temporal cortex, THA = thalamus)

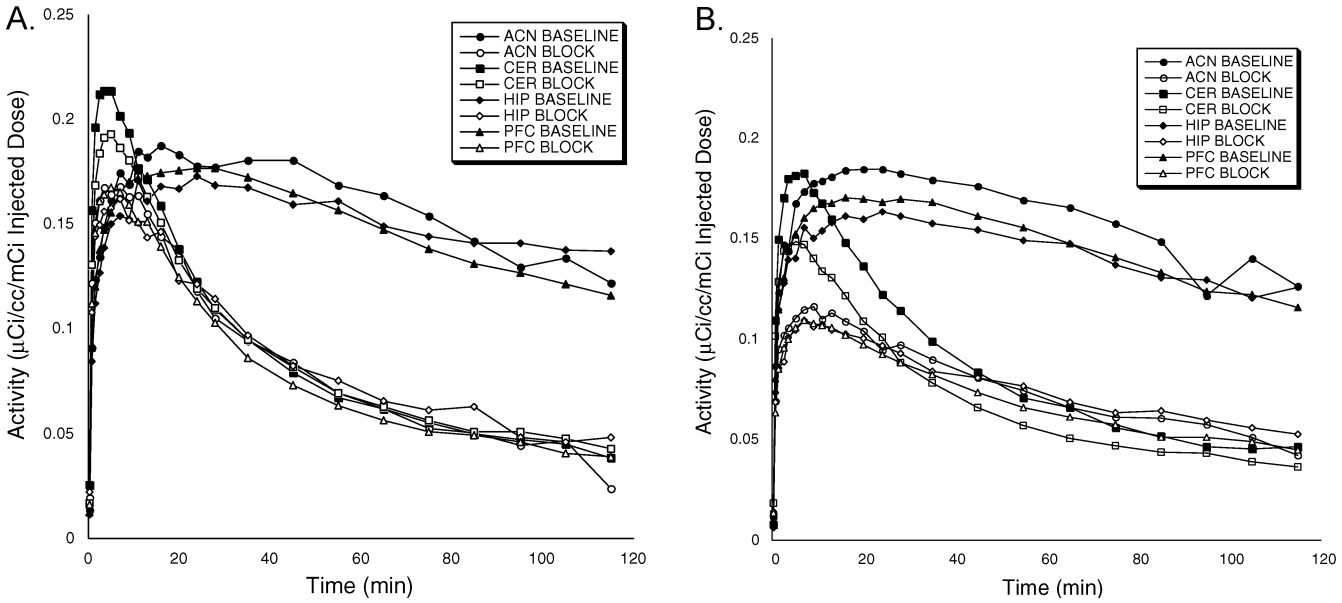


Figure 8. Time−activity curves of the radioactivity in baboon after the injection of [^{11}C]**10**. A. Pretreatment with WAY100635 (0.5 mg/kg iv). B. Pretreatment with 8-OH-DPAT (2 mg/kg i.v). ACN = anterior cingulate, CER = cerebellum, HIP = hippocampus, PFC = prefrontal cortex.

(545 mg, 13.63 mmol, 60 wt %) in DMF (15 mL). After stirring for 30 min at room temperature (rt), the reaction mixture was treated with iodomethane (0.9 mL, 14.45 mmol) and stirred for 6h. After concentrating to dryness under vacuum, the residue was dissolved in ethanol (10 mL) and treated with catalytic amount of *p*-toluenesulfonic acid (PTSA) (250 mg, 1.31 mmol) and then heated for 2h under reflux conditions. The reaction mixture was concentrated under vacuum, treated with water (5 mL), and extracted with chloroform (3 × 15 mL). The organic phases were dried over $MgSO_4$, concentrated and recrystallized from hexane: diethyl ether (90:10) to give the methylated product **2** (934 mg, 60%). **2**: ^{1}H NMR (400 MHz, $CDCl_3$) 7.37 (s, 1H), 3.34 (s, 3H).

2-(4-Chlorobutyl)-4-methyl-2*H*-[1,2,4]triazine-3,5-dione (3). 4-Methyl-2H-[1,2,4]triazine-3,5-dione (**2**, 859 mg, 6.76 mmol) was added in portions to a suspension of NaH (352 mg, 8.79 mmol, 60 wt %) in anhydrous DMF (6 mL). The reaction mixture was stirred at room temperature for 10 min followed by the addition of 1-bromo-4-chlorobutane (1 mL, 8.8 mmol). The mixture was stirred for 12 h at room temperature and poured into crushed ice, extracted with EtOAc (3 × 30 mL). The combined organic layer was washed with brine, dried ($MgSO_4$), concentrated and column chromatographed (hexane: EtOAc) to yield the product **3**[50] (1.27 g, 87%). **3**: ^{1}H NMR ($CDCl_3$, 400 MHz) 7.39 (s, 1H), 4.02 (d, *J* = 8.9 Hz, 2H), 3.58 (d, *J* = 8.5 Hz, 2H), 3.34 (s, 3H), 1.77−1.98 (m, 4H); HRMS calcd for $C_8H_{13}ClN_3O_2$ (MH^+): 218.0696; Found: 218.0696.

8-Piperazin-1-yl-naphthalen-2-ol (7). A suspension of 8-aminonaphthalen-2-ol (**4**, 796 mg, 5 mmol) and dichloride **6** (892 mg, 5 mmol) in PEG-400 (2 mL) was heated under microwave conditions (3 × 10 s) with ample time between the heating to allow the reaction mixture to cool to room temperature. (CAUTION! A copious amount of HCl gas is released). After the evolution of HCl gas subsided, the reaction mixture was triturated with chloroform to precipitate out the crude solid. The solid residue was filtered and washed with chloroform. The product fraction was further recrystallized from chloroform: methanol (80:20) to give the piperazine **7**[51] (524 mg, 46% yield). **7**: ^{1}H NMR ($CDCl_3$, 400 MHz) 7.80 (d, *J* = 11.6 Hz, 1H), 7.62 (d, *J* = 8.1 Hz, 1H), 7.53−7.54 (m, 1H), 7.30 (m, 1H), 7.22 (s, 1H), 7.15−7.20 (m, 1H), 3.60 (t, *J* = 6.8 Hz, 4H), 3.39−3.41 (m, 4H); HRMS calcd for $C_{14}H_{17}N_2O$ (MH^+): 229.1341; Found: 229.1348.

1-(7-Methoxynaphthalen-1-yl)piperazine (8). A suspension of 7-methoxy-naphthalen-1-ylamine (**5**, 871 mg, 5 mmol) and dichloride (892 mg, 5 mmol) in PEG-400 (2 mL) was heated under microwave conditions (3 × 10 s). After the evolution of HCl gas subsided, the reaction mixture was diluted with chloroform, brought to reflux, diluted with hexane and allowed to cool to room temperature. The solid residue was filtered, washed with hexane

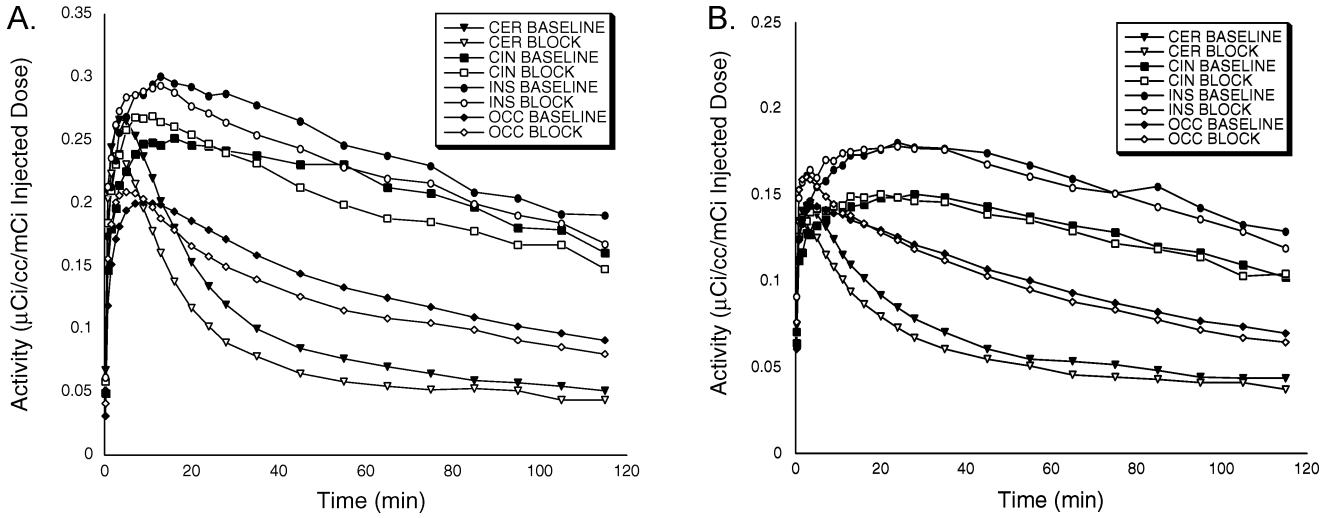


Figure 9. Time activity curves of the radioactivity in baboon after the injection of [^{11}C]**10**. A. Pretreatment with M100907 (2 mg/kg iv). B. Pretreatment with haloperidol (0.25 mg/kg i.v). CIN = cingulate, INS = insula, OCC = occipital cortex, regions with significant densities of 5-HT$_{2A}$ and/or D$_2$ receptors. CER = cerebellum.

and column chromatographed (10% MeOH in $CHCl_3$). The product fraction was further recrystallized from chloroform: hexane (50:50) to give the piperazine **8**[52] (653 mg, 54%). **8**: ^{1}H NMR ($CDCl_3$, 400 MHz) 7.74 (d, J = 11.6 Hz, 1H), 7.56 (d, J = 10.4 Hz, 1H), 7.37 (s, 1H), 7.30−7.23 (m, 1H), 7.15 (d, J = 9.2 Hz, 2H), 3.93 (s, 3H), 3.64−3.22 (m, 8H); HRMS calcd for $C_{15}H_{19}N_2O$-(MH^+): 243.1497; Found: 243.1501.

2-{4-[4-(7-Hydroxynaphthalen-1-yl)piperazin-1-yl]butyl}-4-methyl-2*H*-[1,2,4]triazine-3,5-dione (9). A solution of the piperazine (**7**, 272 mg, 1.19 mmol) and azauracil derivative **3** (151 mg, 0.7 mmol) were dissolved in 1-butanol (4 mL) and treated dropwise with Et_3N (0.5 mL). The reaction mixture was refluxed for 12 h and then concentrated under vacuum. The concentrated solution was triturated with diethyl ether and the resultant off-white solid which precipitated out was filtered, washed with copious amounts of ether and then column chromatographed (5−10% MeOH in $CHCl_3$) to give **9** as a colorless solid (146 mg, 51%). **9**: Mp 205 °C (d); ^{1}H NMR (400 MHz, $CDCl_3$): 7.71 (d, 11.7 Hz, 1H), 7.48−7.45 (m, 2H), 7.37 (s, 1H), 7.22−7.19 (m, 1H), 7.08−7.4 (m, 2H), 4.02 (t, J = 9.5 Hz, 2H), 3.33 (s, 3H), 3.19−3.08 (m, 4H), 2.85−2.70 (m, 4H), 2.54−2.49 (t, J = 10.1 Hz, 2H), 1.87−1.77 (m, 2H), 1.66−1.56 (m, 2H). HRMS calcd for $C_{22}H_{28}N_5O_3$ (MH^+): 410.2192; Found: 410.2194; Analytical HPLC: reverse phase (acetonitrile/0.1 M ammonium formate/40: 60, flow rate: 2 mL/min), t_R: 2.7 min., purity >99%; For the purpose of elemental analyses, a solution of the compound **9** (40 mg, 0.1 mmol) in CH_2Cl_2 (5 mL) under argon was added dropwise to a solution of hydrochloric acid (1M in Et_2O, 100 μL, 0.10 mmol) at 0 °C. After vigorously stirring for 2 h at room temperature, the resultant precipitate was filtered, repeatedly washed with dichloromethane followed by ice-cold methanol (2 × 5 mL) and dried under vacuum to give the corresponding hydrochloride salt (37 mg, 0.083 mmol) in 85% yield. Anal. ($C_{22}H_{27}N_5O_3$.HCl) C, H, N.

2-{4-[4-(7-Methoxy-naphthalen-1-yl)piperazin-1-yl]-butyl}-4-methyl-2*H*-[1,2,4]triazine-3,5-dione (10). A solution of the piperazine (**8**, 272 mg, 1.19 mmol) and azauracil derivative **3** (151 mg, 0.7 mmol) were dissolved in 1-butanol (4 mL) and treated dropwise with Et_3N (0.5 mL). The reaction mixture was refluxed for 12 h and then concentrated under vacuum. The concentrated solution was triturated with diethyl ether, and the resultant off-white solid which precipitated out was filtered, washed with copious amounts of ether, and then column chromatographed (5−10% MeOH in $CHCl_3$) to give **10** as a colorless solid (146 mg, 51%) yield. **10**: Mp: 210 °C (decomp); ^{1}H NMR (400 MHz, $CDCl_3$): 7.73 (d, 8.9 Hz, 1H), 7.52−7.48 (m, 1H), 7.41 (s, 1H), 7.25−7.28 (m, 1H), 7.13 (dd, J = 2.6, 8.9 Hz, 1H), 7.08−7.10 (m, 1H), 4.04 (t, J = 7.2 Hz, 2H), 3.94 (s, 3H), 3.35 (s, 3H), 3.16−3.12 (m, 4H), 2.78−2.74 (m, 4H), 2.52 (t, J = 7.4 Hz, 2H), 1.88−1.80 (m, 2H), 1.65−1.59 (m, 2H). HRMS calcd for $C_{23}H_{30}N_5O_3$ (MH^+): 424.2349; Found: 424.2361. Analytical HPLC: reverse phase (acetonitrile/0.1 M ammonium formate/acetic acid solution 35: 65: 0.5, flow rate: 2 mL/min), t_R: 6.1 min., purity >99%; normal phase Phenomenex, luna silica 4.6 × 250 mm, 5 μ column (ethyl acetate/hexane/diethylamine 90:10:0.2, flow rate 2 mL/min), t_R: 2.5 min, purity >99%. For the purpose of elemental analyses, a solution of the compound **10** (50 mg, 0.118 mmol) in CH_2Cl_2 (5 mL) under argon was added dropwise to a solution of hydrochloric acid (1 M in Et_2O, 120 μL, 0.12 mmol) at 0 °C. After stirring for 30 min at room temperature, the resultant precipitate was filtered, repeatedly washed with dichloromethane followed by ice-cold methanol (2 × 5 mL), and dried under vacuum to give the corresponding hydrochloride salt (42 mg, 0.091 mmol) in 77% yield. Anal. ($C_{23}H_{29}N_5O_3$.HCl) C: calcd, 60.06; found, 59.30. H: calcd, 6.57; found, 6.54. N: calcd, 15.23; found, 14.92.

Radiosynthesis of [^{11}C]10. The precursor **9** (1.0 mg) was dissolved in 400 μL of acetone in a capped 1 mL vial. Aqueous NaOH (10 μL, 5 M) was then added to the solution and was allowed to stand for 5 min. [^{11}C]-Methyl triflate was transported by a stream of argon (20−30 mL/min) into the vial over a period of 5 min at room temperature. At the end of the trapping, the reaction mixture was directly injected into a semipreparative RP-HPLC (Phenomenex C18, 10 × 250 mm, 10 μ) and eluted with acetonitrile: 0.1 M ammonium formate solution (40:60) at a flow rate of 10 mL/min. The precursor appeared at 4−5 min during the semipreparative HPLC analysis. The product fraction with a retention time of 8−9 min based on γ-detector was collected, diluted with 100 mL of deionized water, and passed through a classic C-18 Sep-Pak cartridge. Reconstitution of the product in 1 mL of absolute ethanol afforded [^{11}C]**10** in 25 ± 5% (n = 15) yield based on [^{11}C]CO_2 at EOS. A portion of the ethanol solution was analyzed by analytical HPLC (Phenomenex, Prodigy ODS(3) 4.6 × 250 mm, 5 μ; mobile phase: acetonitrile/0.1 M ammonium formate/acetic acid solution 35: 65: 0.5, flow rate: 2 mL/min, retention time: 6.1 min) to determine the specific activity and radiochemical purity.

Partition Coefficient Measurement. Partition coefficient (log$P$) of the [^{11}C]**10** was measured by mixing 0.1 mL of the radioligand formulation with 5 g each of 1-octanol and freshly prepared PBS buffer (pH = 7.4) in a culture tube.[44] The culture tube was shaken mechanically for 5 min followed by centrifugation for 5 min. Radioactivity per 0.5 g each of 1-octanol and aqueous layer was measured using a well counter. The partition coefficient was determined by calculating the ratio of counts/g of 1-octanol to that of buffer. 1-Octanol fractions were repeatedly portioned with fresh buffer to get consistent values for partition coefficient. All the experimental measurements were performed in triplicate.

In Vitro Agonist Binding Assay. Preparation of Membrane Fractions from CHO-h5-HT$_{1A}$ Cells. Membranes from CHO cells stably expressing the human 5-HT$_{1A}$ receptor at a density of 8 pmol/mg membrane protein with 5-HT$_{2c}$ receptor background were prepared. Cells were grown in DMEM/F-12 medium supplemented with 5% fatal bovine serum, 50 μg/mL geneticin, and 50 μg/mL hygromycin B in a humidified atmosphere of 5% CO_2 until they reached confluence. Cells were harvested by centrifugation (800*g* for 5 min) and homogenized using a polytron homogenizer (Polytron, CH-6010 Kreiens-Lu, Brinkman Instrument, Westbury, NY) in buffer containing 20 mM HEPES, pH7.4, 3 mM $MgCl_2$, and a cocktail of protease inhibitors (Sigma-Aldrich, St. Louis, MO) at 1:2000 dilution. The homogenate was centrifuged (Beckman Optima LE80K Ultracentrifuge) at 100 000*g* for 15 min at 4 °C. The pellet was suspended in the same buffer and recentrifuged. The final pellet was suspended in assay buffer containing 20 mM HEPES, PH 7.4, 3 mM $MgCl_2$, 100 mM NaCl, and a mixture of protease inhibitors and stored at −70 °C. Protein concentration was determined by detergent compatible colorimetric assay using DC Protein Assay Reagents as recommended by the manufacturer (Bio-Rad, Hercules, CA).

Inhibition of [^{3}H]-8-OH-DPAT Binding by 10 or 5-HT in Membranes. For inhibition experiments, the incubation medium consisted of 20 mM HEPES, pH 7.4, 4 mM $CaCl_2$, 5 nM [^{3}H]-8-OH-DPAT, and 30 μg of membrane protein in the presence of different concentrations of **10** or 5-HT. The assay mixture was incubated for 10 min at 30 °C. The reaction was terminated by rapid filtration through Whatman GF/B filters followed by three washes with ice cold 20 mM HEPES buffer, pH 7.4, using a cell harvester (Brandel, M-24R Gaitherburg, MD). Bound radioactivity was determined by liquid scintillation spectrometry (Beckman).

Agonist-Stimulated [^{35}S]GTPγS Binding to Membranes. These experiments were carried out as described previously with some modification.[53] CHO-h5-HT$_{1A}$ membranes (30 μg) were preincubated with agonists for 5 min at room temperature with indicated concentrations in a buffer containing 20 mM HEPES pH 7.4, 3 mM $MgCl_2$, 100 mM NaCl, and 3 μM GDP in a final volume of 0.5 mL. [^{35}S]GTPγS (0.1 nM; 1250 Ci/mmol Perkin-Elmer Life Science, Boston, MA) was added, and the incubation was continued for 60 min at room temperature. Experiments were terminated by rapid filtration through Whatman GF/B filters followed by three washes with ice-cold 20 mM HEPES buffer, pH 7.4, using a cell harvester. Bound radioactivity was determined by liquid scintillation spectrometry.

Assay of Adenylyl Cyclase Activity. These experiments were carried out as described previously with some modification.[54] Cells were cultured in 35 × 10 mm dish until confluent. The day before use, cells were cultured overnight under serum and geneticin/hygromycin B free conditions. Cells were preincubated with DMEM/F-12 containing 100 μM of IBMX for 20 min at 37 °C. Agonist was added at indicated concentrations for 5 min before addition of 10 μM forskolin. The dishes were incubated for 10 min at 37 °C, and the reaction was stopped by aspiration of the medium and addition of 0.1 M HCl. Cellular extract was centrifuged at 14 000*g* for 10 min at 4 °C. Supernatants were diluted to 1:4 and assayed for cAMP levels in duplicate, using a Direct Cyclic AMP enzyme immunoassay Kit (Assay Design, Ann Arbor, MI).

Baboon PET Scanning with [^{11}C]10. All studies were performed in a single male baboon. A fasted animal was induced 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 iv infusion line with 0.9% NaCl was maintained during the experiment and used for hydration and radiotracer injection. An arterial line is placed for obtaining arterial samples for the input function. After a 10 min transmission scan, 5 ± 0.5 mCi of [^{11}C]**10** (specific activity of 1500 ± 300 Ci/mmol) was injected as an iv bolus and emission data were collected for 120 min in 3-D mode in a Siemens ECAT EXACT HR+ (CPS/Knoxville, TN). The head was positioned at the center of the field of view as defined by imbedded laser lines. Regions of interest drawn on the animal's MRI scan were transferred to co-registered (AIR) frames of PET data.

Metabolite Assays. The percentage of radioactivity in plasma as unchanged [^{11}C]**10** was determined by HPLC. Blood samples were taken at 2, 6, 12, 30, 60, and 90 min after radioactivity injection for metabolites analyses. The supernatant liquid obtained after centrifugation of the blood sample at 2000 rpm for 1 min was transferred (0.5 mL) in to 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, the radioactivity was measured in a well-counter, and the majority (0.8 mL) was subsequently injected onto the HPLC column (column: Waters μBondpack-C18 7.8 × 300 mm, 10 μ; mobile phase: 40:60 (acetonitrile:water containing 1% phosphoric acid); flow rate: 5.5 mL/min; retention time of the [^{11}C]**10**: 8 min) equipped with a series 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. To reaffirm that the retention time of the parent has not shifted during the course of the metabolite analysis, a QC sample of [^{11}C]**10** was injected in the beginning and end of the study. The percentage of radioactive parent obtained is used for the measurement of metabolite corrected arterial input functions.

Kinetic Modeling. Quantification of [^{11}C]WAY100635 V_T. Regional distribution volumes of [^{11}C]WAY100635 are derived from kinetic analysis using the arterial input function and a two tissue compartment (2T) model as the general framework.[55]

Quantification of [^{11}C]10 V_T and BP_2. Derivation of [^{11}C]**10** regional distribution volumes (V_T) for the ROI and voxel based analysis was performed using the likelihood approach to the graphical method (LEGA),[56,57] which estimates parameters using standard likelihood theory, removing the bias present when using ordinary least squares. Brain activity was corrected for the contribution of plasma activity assuming a 5% blood volume in the ROIs.[58] Our main outcome measure was the specific to nonspecific equilibrium partition coefficient or BP_2 ($BP_2 = (V_T - V_2)/V_2 = f_2 B_{max}/K_D$) where f_2 is the free fraction of [^{11}C]**10** in the brain, B_{max} is the total number of available receptors, and $1/K_D$ is the affinity. We used BP_2 because f_1 of this compound is not measurable. For clarity and consistency among groups, we use the BP_1 and BP_2 nomenclature as opposed to BP′ and V_3".

Conclusion

We synthesized [^{11}C]**10**, a promising agonist 5-HT$_{1A}$ receptor PET ligand. Competition binding assays in cell lines demonstrated that **10** has high affinity and selectivity for 5-HT$_{1A}$ receptor. Agonist-stimulated [^{35}S]GTPγS binding assay and inhibition of forskolin-stimulated cAMP production indicated that **10** is a 5-HT$_{1A}$ receptor agonist. PET imaging studies in baboons show that [^{11}C]**10** penetrated the BBB and regional brain uptake was proportional to known concentrations of 5-HT$_{1A}$ receptor binding sites. Metabolite studies show that [^{11}C]**10** underwent fast metabolism and 20 ± 3% of unchanged parent was left in the plasma after 30 min postinjection of [^{11}C]**10**. Block studies with WAY100635 and 8-OH-DPAT displaced all detectable specific binding of [^{11}C]**10**, further demonstrating the potential of [^{11}C]**10** to measure 5-HT$_{1A}$ receptor in vivo. Future studies will determine the sensitivity of the ligand to competition by endogenous serotonin and external agonist drugs and the potential of this tracer to measure agonist receptor occupancy. We conclude that this tracer has potential for assessing agonist binding and can assist in dose finding studies for 5-HT$_{1A}$ agonists being developed for therapeutic applications as well as in the study of disease process and therapeutic effects involving the 5-HT$_{1A}$ receptor.

Acknowledgment. This work was supported by a research grant from the National Institutes of Health (P50 MH62185). The authors also thank Dr. Bryan Roth and the NIMH-PDSP program for receptor-transporter assay results.

Supporting Information Available: Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Arango, V.; Underwood, M. D.; Boldrini, M.; Tamir, H.; Kassir, S. A.; Hsiung, S.-C.; Chen, J. J. X.; Mann, J. J. Serotonin 1A Receptors, Serotonin Transporter Binding and Serotonin Transporter mRNA Expression in the Brainstem of Depressed Suicide Victims. *Neuropsychopharmacology* **2001**, *25*, 892−903.

(2) Arango, V.; Huang, Y. Y.; Underwood, M. D.; Mann, J. J. Genetics of the serotonergic system in suicidal behavior. *J. Psychiatr. Res.* **2003**, *37*, 375−386.

(3) Sullivan, G. M.; Oquendo, M. A.; Simpson, N.; Van Heertum, R. L.; Mann, J. J.; Parsey, R. V. Brain serotonin$_{1A}$ receptor binding in depression is related to psychic and somatic anxiety. *Biol. Psychiatry*, in press.

(4) Merlet, I.; Ostrowsky, K.; Costes, N.; Ryvlin, P.; Isnard, J.; Faillenot, I.; Lavenne, F.; Dufournel, D.; Le Bars, D.; Mauguiere, F.,3 5-HT1A receptor binding and intracerebral activity in temporal lobe epilepsy: an [18F]MPPF-PET study. *Brain* **2004**, *127*, 900−913.

(5) Tiihonen, J.; Keski-Rahkonen, A.; Lopponen, M.; Muhonen, M.; Kajander, J.; Allonen, T.; Nagren, K.; Hietala, J.; Rissanen, A.,# Brain serotonin 1A receptor binding in bulimia nervosa. *Biol. Psychiatry* **2004**, *55*, 871−873.

(6) Meltzer, H. Y.; Li, Z.; Kaneda, Y.; Ichikawa, J. Serotonin receptors: their key role in drugs to treat schizophrenia. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **2003**, *27*, 1159−1172.

(7) Bara-Jimenez, W.; Bibbiani, F.; Morris, M. J.; Dimitrova, T.; Sherzai, A.; Mouradian, M. M.; Chase, T. N. Effects of serotonin 5-HT1A agonist in advanced Parkinson's disease. *Mov. Disord.* **2005**.

(8) Lai, M. K. P.; Tsang, S. W. Y.; Francis, P. T.; Esiri, M. M.; Keene, J.; Hope, T.; Chen, C. P. L. H. Reduced serotonin 5-HT1A receptor binding in the temporal cortex correlates with aggressive behavior in Alzheimer disease. *Brain Res.* **2003**, *974*, 82−87.

(9) Frazer, A.; Maayani, S.; Wolfe, B. B. Subtypes of receptors for serotonin. *Annu. Rev. Pharmacol. Toxicol.* **1990**, *30*, 307−348.

(10) Hoyer, D.; Hannon, J. P.; Martin, G. R. Molecular, pharmacological and functional diversity of 5-HT receptors. *Pharmacol., Biochem. Behav.* **2002**, *71*, 533−554.

(11) Hamon, M.; Lanfumey, L.; el Mestikawy, S.; Boni, C.; Miquel, M. C.; Bolanos, F.; Schechter, L.; Gozlan, H. The main features of central 5-HT1 receptors. *Neuropsychopharmacology* **1990**, *3*, 349−360.

(12) Radja, F.; Daval, G.; Hamon, M.; Verge, D. Pharmacological and physicochemical properties of pre-versus postsynaptic 5-hydroxytryptamine1A receptor binding sites in the rat brain: a quantitative autoradiographic study. *J. Neurochem.* **1992**, *58*, 1338−1346.

(13) Meltzer, C. C.; Price, J. C.; Mathis, C. A.; Butters, M. A.; Ziolko, S. K.; Moses-Kolko, E.; Mazumdar, S.; Mulsant, B. H.; Houck, P. R.; Lopresti, B. J.; Weissfeld, L. A.; Reynolds, C. F. Serotonin 1A receptor binding and treatment response in late-life depression. *Neuropsychopharmacology* **2004**, *29*, 2258−2265.

(14) Clawges, H. M.; Depree, K. M.; Parker, E. M.; Graber, S. G. Human 5-HT1 receptor subtypes exhibit distinct G protein coupling behaviors in membranes from Sf9 cells. *Biochemistry* **1997**, *36*, 12930−12938.

(15) Watson, J.; Collin, L.; Ho, M.; Riley, G.; Scott, C.; Selkirk, J. V.; Price, G. W. 5-HT(1A) receptor agonist-antagonist binding affinity difference as a measure of intrinsic activity in recombinant and native tissue systems. *Br. J. Pharmacol.* **2000**, *130*, 1108−1114.

(16) Gozlan, H.; Thibault, S.; Laporte, A. M.; Lima, L.; Hamon, M. The selective 5-HT1A antagonist radioligand [3H]WAY 100635 labels both G-protein-coupled and free 5-HT1A receptors in rat brain membranes. *Eur. J. Pharmacol.* **1995**, *288*, 173−186.

(17) Mongeau, R.; Welner, S. A.; Quirion, R.; Suranyi-Cadotte, B. E. Further evidence for differential affinity states of the serotonin1A receptor in rat hippocampus. *Brain Res.* **1992**, *590*, 229−238.

(18) Burnet, P. W.; Eastwood, S. L.; Harrison, P. J. [3H]WAY-100635 for 5-HT1A receptor autoradiography in human brain: a comparison with [3H]8-OH-DPAT and demonstration of increased binding in the frontal cortex in schizophrenia. *Neurochem. Int.* **1997**, *30*, 565−574.

(19) Kegeles, L. S.; Mann, J. J. In vivo imaging of neurotransmitter systems using radiolabeled receptor ligands. *Neuropsychopharmacology* **1997**, *17*, 293−307.

(20) Cumming, P.; Gillings, N. M.; Jensen, S. B.; Bjarkam, C.; Gjedde, A. Kinetics of the uptake and distribution of the dopamine D(2,3) agonist (R)-N-[1-(11)C]*n*-propylnorapomorphine in brain of healthy and MPTP-treated Gottingen miniature pigs. *Nucl. Med. Biol.* **2003**, *30*, 547−553.

(21) Cumming, P.; Wong, D. F.; Gillings, N.; Hilton, J.; Scheffel, U.; Gjedde, A. Specific binding of [(11)C]raclopride and N-[(3)H]propylnorapomorphine to dopamine receptors in living mouse striatum: occupancy by endogenous dopamine and guanosine triphosphate-free G protein. *J. Cereb. Blood Flow Metab.* **2002**, *22*, 596−604.

(22) Narendran, R.; Hwang, D. R.; Slifstein, M.; Talbot, P. S.; Erritzoe, D.; Huang, Y.; Cooper, T. B.; Martinez, D.; Kegeles, L. S.; Abi-Dargham, A.; Laruelle, M. In vivo vulnerability to competition by endogenous dopamine: comparison of the D2 receptor agonist radiotracer (−)-N-[11C]propyl-norapomorphine ([11C]NPA) with the D2 receptor antagonist radiotracer [11C]-raclopride. *Synapse* **2004**, *52*, 188−208.

(23) Hirsch, S. R.; Kissling, W.; Bauml, J.; Power, A.; O'Connor, R. A 28-week comparison of ziprasidone and haloperidol in outpatients with stable schizophrenia. *J. Clin. Psychiatry* **2002**, *63*, 516−523.

(24) Passchier, J.; van Waarde, A. Visualisation of serotonin-1A (5-HT1A) receptors in the central nervous system. *Eur. J. Nucl. Med.* **2001**, *28*, 113−129.

(25) Tauscher, J.; Verhoeff, N. P. L. G.; Christensen, B. K.; Hussey, D.; Meyer, J. H.; Kecojevic, A.; Javanmard, M.; Kasper, S.; Kapur, S. Serotonin 5-HT1A receptor binding potential declines with age as measured by [11C]WAY-100635 and PET. *Neuropsychopharmacology* **2001**, *24*, 522−530.

(26) Pike, V. W.; Halldin, C.; McCarron, J. A.; Lundkvist, C.; Hirani, E.; Olsson, H.; Hume, S. P.; Karlsson, P.; Osman, S.; Swahn, C.-G.; Hall, H.; Wikstrom, H.; Mensonidas, M.; Poole, K. G.; Farde, L. [Carbonyl-11C]Desmethyl-WAY-100635 (DWAY) is a potent and selective radioligand for central 5-HT1A receptors in vitro and in vivo. *Eur. J. Nucl. Med.* **1998**, *25*, 338−346.

(27) Zimmer, L.; Rbah, L.; Giacomelli, F.; Le Bars, D.; Renaud, B. A reduced extracellular serotonin level increases the 5-HT1A PET ligand 18F-MPPF binding in the rat hippocampus. *J. Nucl. Med.* **2003**, *44*, 1495−1501.

(28) Gozlan, H.; Ponchant, M.; Daval, G.; Verge, D.; Menard, F.; Vanhove, A.; Beaucourt, J. P.; Hamon, M. 125I−Bolton-Hunter-8-methoxy-2-[N-propyl-N-propylamino]tetralin as a new selective radioligand of 5-HT1A sites in the rat brain. In vitro binding and autoradiographic studies. *J. Pharmacol. Exp. Ther.* **1988**, *244*, 751−759.

(29) Ponchant, M.; Beaucourt, J. P.; Vanhove, A.; Daval, G.; Verge, D.; Hamon, M.; Gozlan, H. [125I−BH-8-MeO-N-PAT, a new ligand for the study of 5-HT1A receptors in the central nervous system]. *C. R. Acad. Sci. III.* **1988**, *306*, 147−152.

(30) Zhuang, Z. P.; Kung, M. P.; Kung, H. F. Synthesis of (*R*,*S*)-*trans*-8-hydroxy-2-[*N*-*n*-propyl-*N*-(3′-iodo-2′-propenyl)amino]tetral in (*trans*-8-OH-PIPAT): a new 5-HT1A receptor ligand. *J. Med. Chem.* **1993**, *36*, 3161−3165.

(31) Halldin, C.; Wikstrom, H.; Swahn, C. G.; Sedvall, G.; Stjernlof, P.; Farde, L. Preparation of [*propyl*-11C]OSU 191, a highly potent and selective 5-HT1A agonist for PET [abstract]. *J. Labelled Compd. Radiopharm.* **1994**, *35*, S675−S677.

(32) Suehiro, M.; Underwood, M.; Arango, V.; Wang, T. S.; Kassir, S.; Bakalian, M.; Yatabe, T.; Pratap, M.; Van Heertum, R. L.; Mann, J. J. In vivo biodistribution of a radiotracer for imaging serotonin-1A receptor sites with PET: [11C]LY274601. *Life Sci.* **1998**, *63*, 1533−1542.

(33) Mathis, C. A.; Huang, Y.; Simpson, N. R. Synthesis and evaluation of 5HT1A agonists as radioligands: failure of G protein-coupled receptor agonists as in vivo imaging agents [abstract]. *J. Labelled Compd. Radiopharm.* **1997**, *40*, 563−564.

(34) Thorell, J. O.; Hedberg, M. H.; Johansson, A. M.; Hacksell, U.; Stone-Elander, S.; Eriksson, L.; Ingvar, M. (R)-[N-11C-methyl]-11-hydroxy-10-methylaporphine as a ligand for 5-HT1A receptors: synthesis and evaluation of its biodistribution in monkey with PET [abstract]. *J. Labelled Compd. Radiopharm.* **1995**, *44*, S179.

(35) Barf, T. A.; Visser, G. M.; van Waarde, A.; Korte, S. M.; Postema, F.; Leyssen, D.; Van Delft, A. M.; Wikstrom, H.; Bohus, B.; Korf, J.; Vaalburg, W. Synthesis and biodistribution of [C-11]Org-13502, a high-affinity serotonin (5-HT1A) receptor ligand [abstract]. *J. Nucl. Med.* **1995**, *36*, 163P.

(36) Fujio, M.; Nagata, S.; Kawamura, K.; Sugiyama, N.; Tanaka, H.; Uno, K.; Ishiwata, K. Synthesis and evaluation of 11C-labeled (S)-N-{[1-(2-phenylethyl) pyrrolidin-2-yl]methyl}-3-methylthiobenzamide as a PET 5-HT1A receptor ligand. *Nucl. Med. Biol.* **2002**, *29*, 657−663.

(37) Hwang, D. R.; Ngo, K.; Savenkova, L.; Huang, Y.; Guo, N. N.; Zhu, Z.; Laruelle, M. 1-[2[(4-[F-18]fluorobenzamido-1-ethyl]4-(1,2,3,4-

tetrahydronaphth-1-yl)-piperidine (FBP) [abstract]. *J. Labelled Compd. Radiopharm.* **2001**, *44*, S179.
(38) Vandecapelle, M.; Dumont, F.; De Vos, F.; Strijckmans, K.; Leysen, D.; Audenaert, K.; Dierckx, R. A.; Slegers, G. Synthesis and preliminary in vivo evaluation of 4-[^{18}F]fluoro-N-{2-[4-(6-trifluoromethylpyridin-2-yl)piperazin-1-yl]ethyl}benzamide, a potential PET radioligand for the 5-HT_{1A} receptor. *J. Labelled Compd. Radiopharm.* **2004**, *47*, 531−542.
(39) Vandecapelle, M.; De Vos, F.; Vermeirsch, H.; De Ley, G.; Audenaert, K.; Leysen, D.; Dierckx, R. A.; Slegers, G. In vivo evaluation of 4-[123I]iodo-N-[2[4-(6-trifluoromethyl-2-pyridinyl)-1-piperazinyl]ethyl]benzamide, a potential SPECT radioligand for the 5-HT1A receptor. *Nucl. Med. Biol.* **2001**, *28*, 639−643.
(40) Zimmer, L.; Fournet, G.; Joseph, B.; Guillaumet, G.; Le Bars, D. Carbon-11 labelling of 8{{3-[4-(2-[11C]methoxyphenyl)piperazin-1-yl]-2-hydroxypropyl}oxy}thiochroman, a presynaptic 5-HT1A receptor agonist, and its in vivo evaluation in anaesthetised rat and in awake cat. *Nucl. Med. Biol*. **2003**, *30*, 541−546.
(41) Carson, R. E.; Kiesewetter, D. O.; Jagoda, E.; Der, M. G.; Herscovitch, P.; Eckelman, W. C. Muscarinic cholinergic receptor measurements with [18F]FP-TZTP: control and competition studies. *J. Cereb. Blood Flow Metab*. **1998**, *18*, 1130−1142.
(42) Bruins Slot, L. A.; Koek, W.; Tarayre, J.-P.; Colpaert, F. C. Tolerance and inverse tolerance to the hyperalgesic and analgesic actions, respectively, of the novel analgesic, F 13640. *Eur. J. Pharmacol*. **2003**, *466*, 271−279.
(43) Koek, W.; Vacher, B.; Cosi, C.; Assie, M. B.; Patoiseau, J. F.; Pauwels, P. J.; Colpaert, F. C. 5-HT1A receptor activation and antidepressant-like effects: F 13714 has high efficacy and marked antidepressant potential. *Eur. J. Pharmacol.* **2001**, *420*, 103−112.
(44) Wilson, A. A.; Jin, L.; Garcia, A.; DaSilva, J. N.; Houle, S., An admonition when measuring the lipophilicity of radiotracers using counting techniques. *Appl. Radiat. Isot.* **2001**, *54*, 203−208.
(45) Langer, O.; Halldin, C.; Chou, Y.; Sandell, J.; Swahn, C.; Nagren, K.; Perrone, R.; Berardi, F.; Leopoldo, M.; Farde, L., Carbon-11 pb-12: an attempt to visualize the dopamine d(4) receptor in the primate brain with positron emission tomography. *Nucl. Med. Biol.* **2000**, *27*, 707−714.
(46) Zhang, M. R.; Haradahira, T.; Maeda, J.; Okauchi, T.; Kawabe, K.; Noguchi, J.; Kida, T.; Suzuki, K.; Suhara, T., Syntheses and pharmacological evaluation of two potent antagonists for dopamine D4 receptors: [11C]YM-50001 and N-[2-[4-(4-Chlorophenyl)-piperizin-1-yl]ethyl]-3-[11C]methoxybenzamide. *Nucl. Med. Biol.* **2002**, *29*, 233−241.
(47) Thomas, D. R.; Atkinson, P. J.; Hastie, P. G.; Roberts, J. C.; Middlemiss, D. N.; Price, G. W., [3H]-SB-269970 radiolabels 5-HT7 receptors in rodent, pig and primate brain tissues. *Neuropharmacology* **2002**, *42*, 74−81.
(48) Hall, H.; Lundkvist, C.; Halldin, C.; Farde, L.; Pike, V. W.; McCarron, J. A.; Fletcher, A.; Cliffe, I. A.; Barf, T.; Wikstroem, H.; Sedvall, G., Autoradiographic localization of 5-HT1A receptors in the post-mortem human brain using [3H]WAY-100635 and [11C]-WAY-100635. *Brain Res.* **1997**, *745*, 96−108.
(49) Kumar, J. S. D.; Prabhakaran, J.; Arango, V.; Parsey, R. V.; Underwood, M. D.; Simpson, N. R.; Kassir, S. A.; Majo, V. J.; Van Heertum, R. L.; Mann, J. J. Synthesis of [O-methyl-11C]1-(2-chlorophenyl)-5-(4-methoxyphenyl)-4-methyl-1H-pyrazole-3-carboxylic acid piperidin-1-ylamide: a potential PET ligand for CB1 receptors. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2393−2396.
(50) Patoiseau, J-. F.; Faure, C.; Dupont-Passelaigue, E.; Couret, F.; Koek, W. Novel 3,5-dioxo-(2H,4H)-1,2,4-triazine derivatives, their preparation and use as drugs**.** PCT Int. Appl. 1996, WO 9616949, 32 pp.
(51) Poindexter, G. S.; Bruce, M. A.; LeBoulluec, K. L.; Monkovic, I. Use of 2-oxazolidinones as latent aziridine equivalents. III. Preparation of N-substituted piperazines. *Tetrahedron Lett.* **1994**, *35*, 7331−7334.
(52) Vassal, T.; Peynot, M. C.; Allen, J.; Thenot, J. P.; Manoury, P.; Sevrin, M.; George, P. Preparation of 1-[2-(1H-inden-3-yl)ethyl]-4-naphthalen-1-ylpiperazines and their affinity for serotoninergic receptors. *Fr. Demande* **1997**, 23.
(53) Newman-Tancredi, A.; Cussac, D.; Marini, L.; Millan, M. J. Antibody capture assay reveals bell-shaped concentration−response isotherms for h5-HT1A receptor-mediated Gai3 activation: Conformational selection by high-efficacy agonists, and relationship to trafficking of receptor signaling. *Mol. Pharmacol*. **2002**, *62*, 590−601.
(54) Mendez, J.; Kadia, T. M.; Somayazula, R. K.; El-Badawi, K. I.; Cowen, D. S. Differential coupling of serotonin 5-HT1A and 5-HT1B receptors to activation of ERK2 and inhibition of adenylyl cyclase in transfected CHO cells. *J. Neurochem.* **1999**, *73*, 162−168.
(55) Parsey, R. V.; Slifstein, M.; Hwang, D.-R.; Abi-Dargham, A.; Simpson, N.; Mawlawi, O.; Guo, N.-N.; Van Heertum, R.; Mann, J. J.; Laruelle, M. Validation and reproducibility of measurement of 5-HT1A receptor parameters with [carbonyl-11C]WAY-100635 in humans: Comparison of arterial and reference tissue input functions. *J. Cereb. Blood Flow Metab.* **2000**, *20*, 1111−1133.
(56) Ogden, R. T. On estimation of kinetic parameters in graphical analysis of PET imaging data. *Stat. Med.* **2003**, *22*, 3557−3568.
(57) Parsey, R. V.; Ogden, R. T.; Mann, J. J. Determination of Volume of Distribution using Likelihood Estimation in Graphical Analysis: Elimination of Estimation Bias. *J. Cereb. Blood Flow Metab.* **2003**, *23*, 1471−1478.
(58) Mintun, M. A.; Raichle, M. E.; Kilbourn, M. R.; Wooten, G. F.; Welch, M. J. A quantitative model for the in vivo assessment of drug binding sites with positron emission tomography. *Ann. Neurol.* **1984**, *15*, 217−227.

JM050725J