Development of Fluorine-18-Labeled 5-HT_{1A} Antagonists

Lixin Lang,* Elaine Jagoda, Bernard Schmall, Bik-Kee Vuong, H. Richard Adams, David L. Nelson,† Richard E. Carson, and William C. Eckelman

Positron Emission Tomography Department, Clinical Center, National Institutes of Health, 10 Center Drive, Bethesda, Maryland 20892, and Eli Lilly & Company, Indianapolis, Indiana 46285

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We have synthesized five fluorinated derivatives of WAY 100635, *N*-{2-[4-(2-methoxyphenyl)-piperazino]ethyl}-*N*-(2-pyridyl)cyclohexanecarboxamide (**4a**), using various acids in place of the cyclohexanecarboxylic acid (CHCA, **2a**) in the reaction scheme. The five acids are 4-fluorobenzoic acid (FB, **2b**), 4-fluoro-3-methylbenzoic acid (MeFB, **2c**), *trans*-4-fluorocyclohexanecarboxylic acid (FC, **2d**), 4-(fluoromethyl)benzoic acid (FMeB, **2e**), and 3-nitro-4-(fluoromethyl)benzoic acid (NFMeB, **2f**) (see Scheme 1). These compounds were radiolabeled with fluorine-18, and their biological properties were evaluated in rats and compared with those of [^{11}C]carbonyl WAY 100635 ([*carbonyl*-^{11}C]**4a**). [*Carbonyl*-^{11}C]**4a** cleared the brain with a biological half-life averaging 41 min. The metabolite-corrected blood radioactivity had a half-life of 29 min. [^{18}F]FCWAY ([^{18}F]**4d)** gave half-lives and intercepts comparable to [*carbonyl*-^{11}C]**4a** in the brain, but the blood clearance was faster. [^{18}F]FBWAY ([^{18}F]**4b**) showed an early rapid net efflux from the whole brain, clearing with a biological half-life of 35 min. The metabolite-corrected blood half-life was 41 min. The comparable whole brain and blood half-lives for Me[^{18}F]FBWAY ([^{18}F]**4c**) were 16 and 18 min, respectively. For each compound, the corresponding carboxylic acid was identified as a major metabolite in blood. Fluoride was also found after injection of [^{18}F]**4d**. However, for all compounds there was a good correlation ($R >$ 0.97) between the differential uptake ratio (DUR, (%ID/g) × body weight (g)/100) in individual rat brain regions at 30 min after injection and the concentration of receptors as determined by in vitro quantitative autoradiography in rat. Specific binding ratios [region of interest (ROI)/cerebellum−1] in control studies for cortex (Ctx) and hippocampus (H) were higher for [*carbonyl*-^{11}C]**4a** and [^{18}F]**4d** compared to [^{18}F]**4b** and [^{18}F]**4c**. [^{18}F]**4d** has similar pharmacokinetic properties and comparable specific binding ratios to [*carbonyl*-^{11}C]**4a**. Fifty nanomoles of **4a** blocked only 30% of the specific binding of [^{18}F]**4d**, while complete blockade was obtained from co-injection of 200 nmol of **4a** (H/Cb-1 from 17.2 to 0.6). [^{18}F]**4b** and [^{18}F]**4c** showed lower specific binding ratios than [*carbonyl*-^{11}C]**4a** and [^{18}F]**4d**. [^{18}F]**4c** was superior to [^{18}F]**4b** since its specific binding was more readily blocked by **4a**. These studies suggest that [^{18}F]**4c** should be a useful compound to assess dynamic changes in serotonin levels while [^{18}F]**4d**, with its high contrast and F-18 label, should provide better statistics and quantification for static measurement of 5-HT_{1A} receptor distribution.

Introduction

Serotonin receptors, specifically the 5-HT_{1A} subtype, have been implicated in anxiety, dementia, schizophrenia, and depression.[1] It has been difficult to fully isolate the effects of the 5-HT_{1A} system because of the large number of 5-HT receptor subtypes[2] and the difficulty in defining the agonist and antagonist properties of potential subtype-selective ligands using physiological models.[1] Studies with the 5-HT_{1A} receptor agonist 8-OH-DPAT [8-hydroxy-2-(di-*n*-propylamino)tetralin] showed it to be selective for this subtype and defined many functional responses that are believed to be a result of 5-HT_{1A} receptor activation. However, many of the antagonists used to date (spiperone, propanolol, and pindolol) are nonselective, and some of the ligands that were initially characterized as antagonists (BMY7378, spiroxatrine, NAN190, UH301, SDZ216525, S14063) were subsequently shown to have partial agonist properties and often high affinity to other biogenic amine receptor systems. Clearly, a highly selective antagonist is needed to characterize this receptor subtype. The term "silent antagonist" has been introduced to distinguish true 5-HT_{1A} receptor antagonists from those that may also be partial agonists. In addition, most of these ligands showed relatively weak binding to the 5-HT_{1A} receptor and cannot be used for studies using radioligands in vivo with positron emission tomography (PET).

In 1992, Crouzel et al. reviewed ligands and tracers for PET studies of the 5-HT system.[3] The first selective 5-HT_{1A} receptor silent antagonist, WAY 100135 (*N*-*tert*-butyl-3-[4-(2-methoxyphenyl)-1-piperazinyl]-2-phenylpropanamide), has an IC_{50} of 15 nM.[4] The first high-affinity, subtype-selective, silent antagonist appears to be WAY 100635 (**4a**).[5] The carboxamide derivative was radiolabeled with ^{123}I by Kung et al. and shown to have appropriate properties for in vivo imaging.[6] Laporte et al. showed that the tritiated **4a** had the appropriate binding characteristics in the mouse brain,[7] whereas Hume et al. showed that the same compound had the

* Corresponding author: Bldg 10, Rm 1C497, NIH, 10 Center Dr, MSC1180, Bethesda, MD 20892.
† Eli Lilly & Co.

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appropriate distribution in the rat brain.[8] In the latter paper, binding potentials were derived and correlated with the receptor concentration as determined using in vitro studies. In addition, Mathis et al. studied [*O-methyl*-^{11}C]**4a** in rat and rhesus monkeys.[9] In the monkey, C-11 radioactivity increased in the frontal cortex up to 20 min and then slowly cleared. The cortex-to-cerebellum ratio reached a maximum of 5.5. However, Osman et al. showed that a radioactive metabolite of [*O-methyl*-^{11}C]**4a** crossed the blood−brain barrier but did not bind to the 5-HT$_{1A}$ receptor.[10,11] This radioactive metabolite was identified as [*O-methyl*-^{11}C]WAY 100634, *N*-{2-[4-(2-methoxyphenyl)piperazino]ethyl}-2-pyridinamine (**1**). As a result, further labeling attempts concentrated on incorporating the C-11 or F-18 in the carboxamide moiety. The first approach was to radiolabel **4a** at the carbonyl carbon of the cyclohexanecarboxamide.[12] This compound showed reduced nonspecific binding and higher target-to-nontarget ratios and gave only radiolabeled carboxylic acid metabolites. Preliminary PET experiments in our laboratory showed that transient equilibrium was not reached in tissues with the highest specific binding using [*carbonyl*-^{11}C]**4a** in rhesus monkeys. Furthermore, receptor parameter quantification was sensitive to accurate plasma metabolite correction, leading to the use of a reference tissue method.[13] Because of its longer half-life, we therefore investigated the possibility of using a F-18-radiolabeled silent antagonist with comparable or slightly weaker affinity for the 5-HT$_{1A}$ receptor. We concentrated on labeling the analogues of **4a** at the carboxamide moiety in order to minimize metabolites that cross the blood−brain barrier.

Zhuang et al. had shown that various benzoyl substituents affected the K_i of the compound as measured in rat hippocampal homogenates.[14] WAY 100635 (**4a**) had a K_i of 0.84 nM, whereas the *p*-fluorobenzoyl analogue had a K_i of 3.3 nM and the *p*-iodobenzoyl analogue had a K_i of 2.6 nM. We have previously reported that **4a** and the 4-fluorobenzoyl analogue (FBWAY, **4b**) in which the cyclohexane carbonyl was replaced by the 4-fluorobenzoyl moiety have high affinity for the 5-HT$_{1A}$ receptor.[15] We found using an in vitro assay (NovaScreen) that **4a** had a K_i of 0.98 nM for the 5-HT$_{1A}$ receptor, 578 nM for the 5HT$_2$ receptor, and 86.8 nM for the dopamine D$_2$ receptor, which was the highest binding non-5-HT biogenic amine receptor. The **4b** had a K_i of 1.2 nM for the 5-HT$_{1A}$ receptor, 130 nM for the 5-HT$_2$ receptor, and 151 nM for the α_1-adrenoceptor, which was the highest binding non-5-HT biogenic amine receptor for this compound. In addition, Thielen and Frazer concluded that **4b** was a silent antagonist in at least two functional measures of postsynaptic 5-HT$_{1A}$ receptor activation.[16] Recently, Forster et al. have carried out a complete pharmacological profile of **4a**.[17] In a radioligand binding assay, **4a** had a high affinity (pIC$_{50}$ = 8.87) with good specificity, with α_1-adrenoceptor binding being the strongest at a pIC$_{50}$ of only 6.64. They also carried out a number of physiological tests that showed the antagonism of **4a**. In the guinea pig ileum, **4a** antagonized the 5-HT$_{1A}$ receptor-mediated inhibition of electrically evoked twitch. WAY 100635 (**4a)** also inhibited dorsal raphe nucleus 5-HT neuronal firing induced by 8-OH-DPAT. In addition, **4a** did not evoke any component of the 5-HT syndrome or induced hypothermia elicited by agonists. Forster et al. concluded from these experiments that **4a** was a silent antagonist, a term which distinguishes true antagonist from partial agonist.

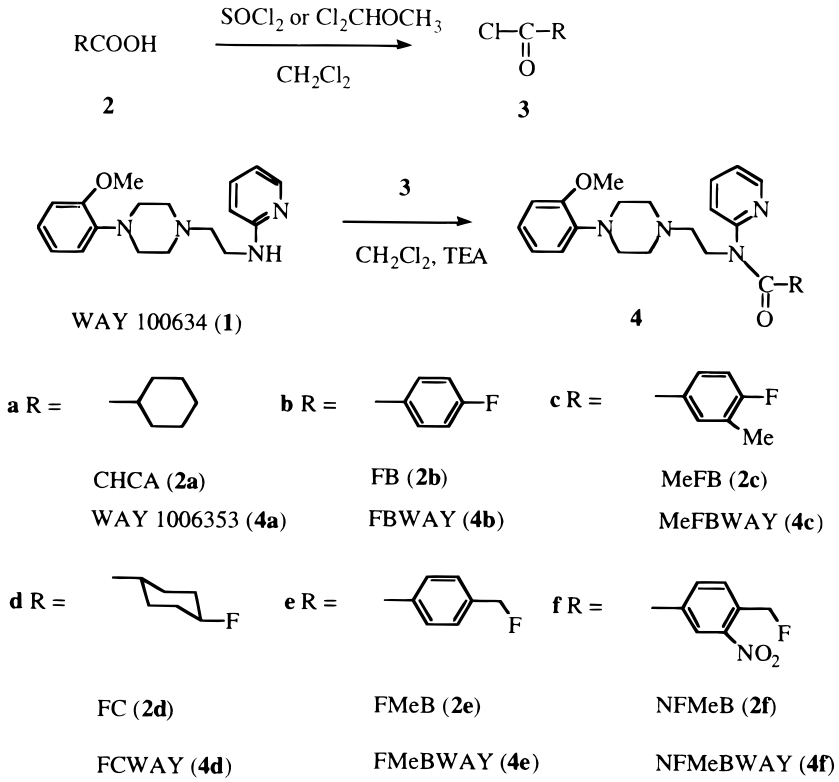
As a result of these preliminary data, we have synthesized four additional derivatives of **4a** using various acids to replace the cyclohexanecarboxylic acid moiety in the reaction scheme. The four acids are 4-fluoro-3-methylbenzoic acid (MeFB, **2c**), 4-(fluoromethyl)benzoic acid (FMeB, **2e**), 3-nitro-4-(fluoromethyl)-benzoic acid (NFMeB, **2f**), and *trans*-4-fluorocyclohexanecarboxylic acid (FC, **2d**) (Scheme 1).[18] The comparable *N*-{2-[4-(2-methoxyphenyl)piperazino]ethyl}-*N*-(2-pyridyl)-cyclohexanecarboxamide is abbreviated by adding WAY to the abbreviation: e.g., the amide resulting from the reaction of 4-fluoro-3-methylbenzoic acid is abbreviated as MeFBWAY.) For comparison, [*carbonyl*-^{11}C]**4a** was also synthesized by radiolabeling in the carbonyl position with C-11 using the procedure of Pike et al.[19] We first determined the metabolite profile in rat brain and blood and then studied saturable binding for those compounds shown to have minimal concentration of metabolites in the rat brain.

Results and Discussion

Chemistry. Reference compounds were prepared for the five fluorinated analogues according Scheme 1. Compounds **4a**−**4e** were prepared by reacting **1** with the corresponding acid chloride **3a**−**3e** in methylene chloride. Each compound was purified and analyzed by elemental analysis, proton NMR, and MS and found to be a single compound of the proposed formula. Among these five compounds, compound **4d** was particularly difficult to make due to the low yield of 4-fluorocyclohexanecarboxylic acid (**2d**) with a six-step reaction sequence. The cyclohexene derivative is the major product during the fluorination process with <10% of the products as the fluoro compound.

Compounds **6a**−**6c** were prepared by coupling glycine sodium salt with the corresponding acid chloride as the reference compounds of possible metabolites. Five substrates (**5b**−**5f**) for radiolabeling were prepared according to Scheme 2. The acids were protected with pentamethylbenzyl ester from either pentamethylbenzyl chloride or pentamethylbenzyl alcohol. This protecting group can be easily removed with a small amount of trifluoroacetic acid during the radiolabeling process. Compounds **5e** and **5f** prepared from pentamethylbenzyl alcohol and 1,3-dicyclohexylcarbodiimide had relatively low yields compared to compounds **7** and **8** due to the formation of anhydrides. Compounds **5b** and **5c** are two very stable triflate salts prepared from **7** and **8** with trifluoromethanesulfonate. Extra care is required for preparing these two substrates since a trace amount of acid can cause the hydrolysis of the protecting group. Compound **5d** was prepared from **9** with 4-nitrobenzenesulfonyl chloride. Mesylate and tosylate derivatives were also prepared from **9**, but the radiolabeling efficiency for mesylate and tosylate was much lower than that of **5d** (<5%). Compound **9** was prepared starting from ethyl 4-hydroxycyclohexanecarboxylate which is a mixture of cis and trans isomers. The ethyl ester (cis and trans mixture) was first hydrolyzed to the acid with

Scheme 1



base and then converted to pentamethylbenzyl ester (cis and trans mixture) using pentamethylbenzyl chloride. There are two distinct proton NMR signals of this mixture for proton at the 4 position of the cyclohexane ring with chemical shifts at 3.6 and 3.9 ppm, respectively.

The mixture was recrystallized using methylene chloride/hexane to give pure **9** with a chemical shift at 3.9 ppm for the proton at the 4 position. Compound **9** can be converted to lactone **8** indicating that this compound is a cis isomer. Compound **10** was prepared from **9** using diethylaminosulfur trifluoride with inversion of configuration to give the trans isomer.

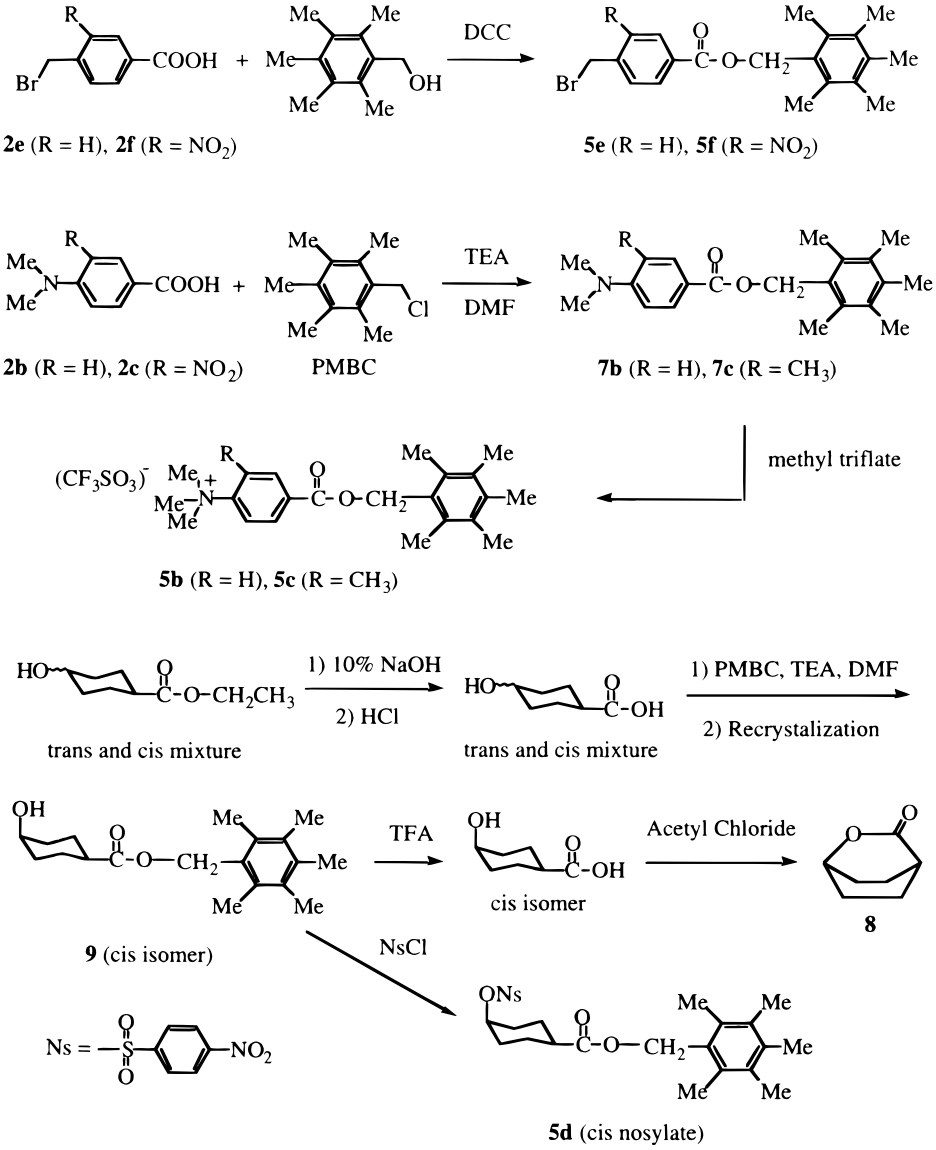
Radiochemistry. Radiolabeling was carried out using F-18 produced by the $^{18}O(p,n)$ reaction. The two fluoromethylbenzoic acid derivatives were radiolabeled using the bromide precursor (**5e** and **5f**),[20] and the two fluorobenzoic acid derivatives were radiolabeled using the trimethylammonium precursor (**5b** and **5c**) following standard procedures (Scheme 3).[21] [^{18}F]**2d** was prepared using the 4-*cis*-nosylate derivative of the pentamethylbenzyl ester of cyclohexanecarboxylic acid (**5d**). After radiolabeling the benzoic acid or the carboxylic acid with F-18, the acid chloride was prepared and reacted with the amine intermediate, WAY 100634 (**1**). Shiue et al.[22] previously radiolabeled the nitro precursor of **4b**, but we obtained low radiochemical yields using their one-step method to make [^{18}F]**4c**. All radiolabeled products were purified by HPLC with radiochemical yields ranging 10−20% and total synthesis time of 80−90 min. The specific activity, determined by using on-line measurements of radioactivity and UV absorption, was >1000 Ci/mmol (EOB) for the F-18 compounds and >250 Ci/mmol (EOB) for the C-11 compounds. The retention times in minutes for the compounds on the same reversed-phase HPLC system with 65% methanol and 0.1 M ammonium formate (**4a** = 30, **4c** = 29, **4b** = 19, **4d** = 18, and **4e** = 16) give an indication of their relative lipophilicity. In this chromatography system **4d** appears to be more polar than **4a** and **4c**.

In Vitro Assays. Three compounds (**4a**, **4c**, and **4d**) were analyzed by various methods to determine the inhibition constant (K_i). The K_i was determined by the inhibition of G-protein activation for all three compounds (Table 1, column 1). In addition, compounds **4a** and **4c** were studied in duplicate in separate studies. For all studies, the affinity of **4a** was similar to that obtained by **4d**, whereas **4c** had a weaker affinity. Compound **4c** also showed α_1 binding with a K_i of 13.2 ± 3.7 nM.

Similar rank order was obtained when competitive binding assays were used (Table 1, columns 2, 3). The MDS Panlabs assays agreed with the previous data obtained by other methods. The data from the competitive assay (column 2) was carried out using cloned cells, as was the competitive assay (column 3). In general, compound **4d** has a stronger binding affinity than compound **4c** by a factor of 3−8. FCWAY (**4d**) had an affinity similar to that obtained for WAY100635.

Metabolites. The chemical form of the radioactive compound in brain and blood was determined by solvent extraction and TLC. In these metabolite studies of brain and blood in rat, we proceeded with testing of an analogue if: (1) the ex vivo extraction efficiency did not decrease with time and on average ≥85% of the radioactivity present in the brain was present in the acetonitrile:water solution and (2) >95% of the extracted radioactivity in the brain was present as the injected compound. The percentage of radioactivity for both [^{18}F]-FMeBWAY ([^{18}F]**4e**) and N[^{18}F]FMeBWAY ([^{18}F]**4f**) in acetonitrile from brain tissue was less than 77% at 5

Scheme 2



min and decreased with time (<44% at 30 min). The mechanism for this retention has not been elucidated although low extraction of fluoride is most likely. Therefore, further studies were not pursued. For all five radiolabeled compounds, the chemical form of the extracted material in whole brain was >95% parent compound from 5 to 30 min. Because of the small percentage difference, no correction was made on the reported DUR values for brain (Figure 1).

The radioactivity in the blood was extracted at high efficiencies for Me[^{18}F]FBWAY ([^{18}F]**4c**) (83−90%), [^{18}F]-FBWAY ([^{18}F]**4b**) (84−92%), and [^{11}C]**4a** (90−97%), whereas for [^{18}F]FCWAY ([^{18}F]**4d**) the extraction decreased from 65% to 33% from 5 to 30 min, most likely due to the percent fluoride present.

For Me[^{18}F]FB ([^{18}F]**2c**), a metabolite of Me[^{18}F]-FBWAY ([^{18}F]**4c**), the extraction from brain tissue was between 72% and 80%, whereas the blood extraction efficiency was between 80% and 85%. For the analogous metabolite of [^{18}F]FCWAY ([^{18}F]**4d**), [^{18}F]FC ([^{18}F]**2d**), the extraction from brain and blood was below 85%, presumably because of the rapid defluorination of this metabolite. The values for both brain and blood were corrected for extraction efficiency assuming that the radioactivity remaining in the pellet was in the form of fluoride (Figure 2b). Fluoride is extracted with acetonitrile at an efficiency of 18 ± 5% in blood between 5 and 30 min after injection of pure F-18 fluoride, whereas the extraction from brain tissue is 5 ± 0.7% under the same conditions (n = 8). Since fluoride does not cross the blood−brain barrier to an appreciable extent, the fluoride observed in brain tissue in this experiment is most likely due to intravascular radioactivity.

Biodistribution. The radioactivity in brain and blood samples is presented in terms of the DUR [(%ID/g) × body weight (g)/100] in order to normalize for the differences in rat weight (Figures 1 and 2). The corresponding carboxylic acid and other more polar metabolites were found in blood in all studies. For **4a** (Figure 1a) the radioactivity in whole brain was maximal at the first time point (5 min) and decreased over time with a biological half-life averaging 41 min. The parent compound had a half-life of 29 min in blood (Figure 1a). The ratio of the brain to metabolite-corrected blood (~4.2) was lower than previously observed[7] most likely because of the relatively high mass injected (see Experimental

Scheme 3

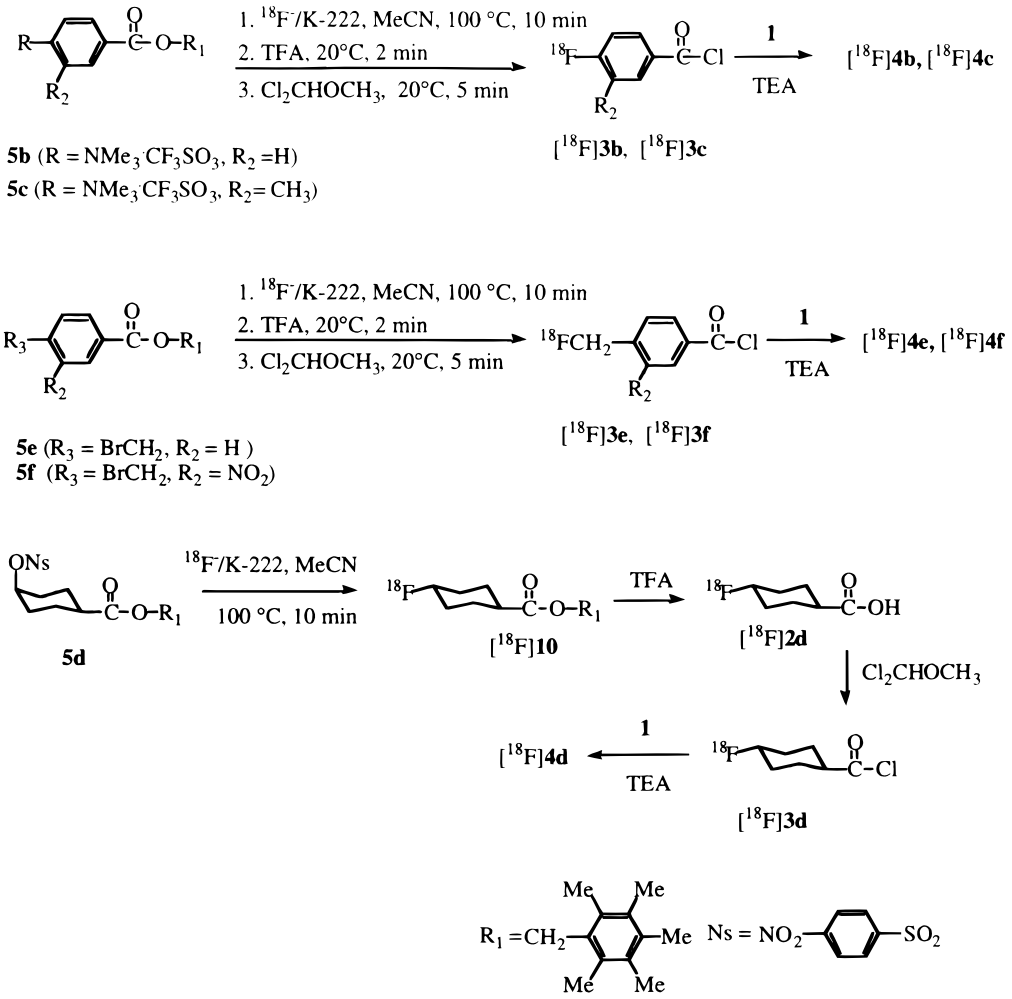


Table 1. Inhibition Constants (K_i, nM) Obtained by in Vitro Assay

compound	5-HT GTP-γ-S	K_i vs [^{3}H]8-OH-DPAT	MDS Panlabs assay
WAY 100635 (**4a**)	1.09 (n = 2) 0.34 ± 0.06	0.59 (n = 2)	
MeFBWAY (**4c**)	4.02 (n = 2) 2.9 ± 0.35	3.96 (n = 2) 1.14 ± 0.36	0.791 ± 0.113
FCWAY (**4d**)	1.04 ± 0.08	0.515 ± 0.047	0.247 ± 0.085

Section). [^{18}F]FBWAY ([^{18}F]**4b**) had an early rapid net efflux (Figure 1b). The second component of the whole brain clearance has a biological half-life of 35 min with high uptake and a blood clearance of 41 min. The comparable whole brain and blood half-lives for Me[^{18}F]-FBWAY ([^{18}F]**4c**) were shorter, i.e., 16 and 18 min, respectively (Figure 1c). The [^{18}F]FCWAY ([^{18}F]**4d**) gave a brain half-life comparable to [^{11}C]**4a** (Figure 1d). The blood half-time was faster (16 min).

The biodistribution of the radiofluorinated metabolites was also studied in the same paradigm using TLC conditions that separated the benzoic acid from metabolites (most likely the glycine conjugates) to determine the pharmacokinetics and the blood−brain barrier penetration of these metabolites. The fluorobenzoic acid metabolites of Me[^{18}F]FBWAY ([^{18}F]**4c**) and [^{18}F]FB-WAY ([^{18}F]**4d**), i.e., Me[^{18}F]FB (Figure 2a) and [^{18}F]FB (data not shown), respectively, both cleared rapidly from the brain and the blood with the brain concentration being substantially lower than the blood concentration. For FCWAY (**4d**), the major metabolite, *trans*-4-fluorocyclohexanecarboxylic acid (**2d**) and its metabolites, most likely the glycine conjugates and fluoride, also showed minimal uptake in the brain (compared to FCWAY, **4d**) and cleared with a similar half-life from blood and brain (Figure 2b). Note that, unlike Me[^{18}F]-FB ([^{18}F]**2c**), the brain-to-blood ratio for [^{18}F]FC ([^{18}F]-**2d**) is approximately 1.0, demonstrating uptake into brain tissue. The pK_a of the carboxylic acids is in the range of 4.2−4.9; thus at physiological pH, the concentration ratio of charged to uncharged molecules is approximately 1000:1. However, the uncharged molecules presumably cross the blood−brain barrier, eventually resulting in equilibrium between brain and blood.[23] We have previously reported brain localization in monkey studies using [^{11}C]CHCA ([^{11}C]**2a**).[24]

Recently, there have been other analogues of **4a** prepared for use as radiolabeled silent antagonists. Zhuang et al.[25] prepared cyclic amides in an attempt to alter the metabolism, but this led to non-5-HT_{1A} receptor binding in vivo. Wilson et al.,[26] on the basis of metabolism studies using a liver cytosol and microsomal preparation of human liver, chose the [2.2.2]bicyclooctane-1-carboxylic acid as the compound with the least metabolism to the carboxylic acid. However, there was extensive metabolism to an unidentified compound that was identical whether the compound was labeled in the

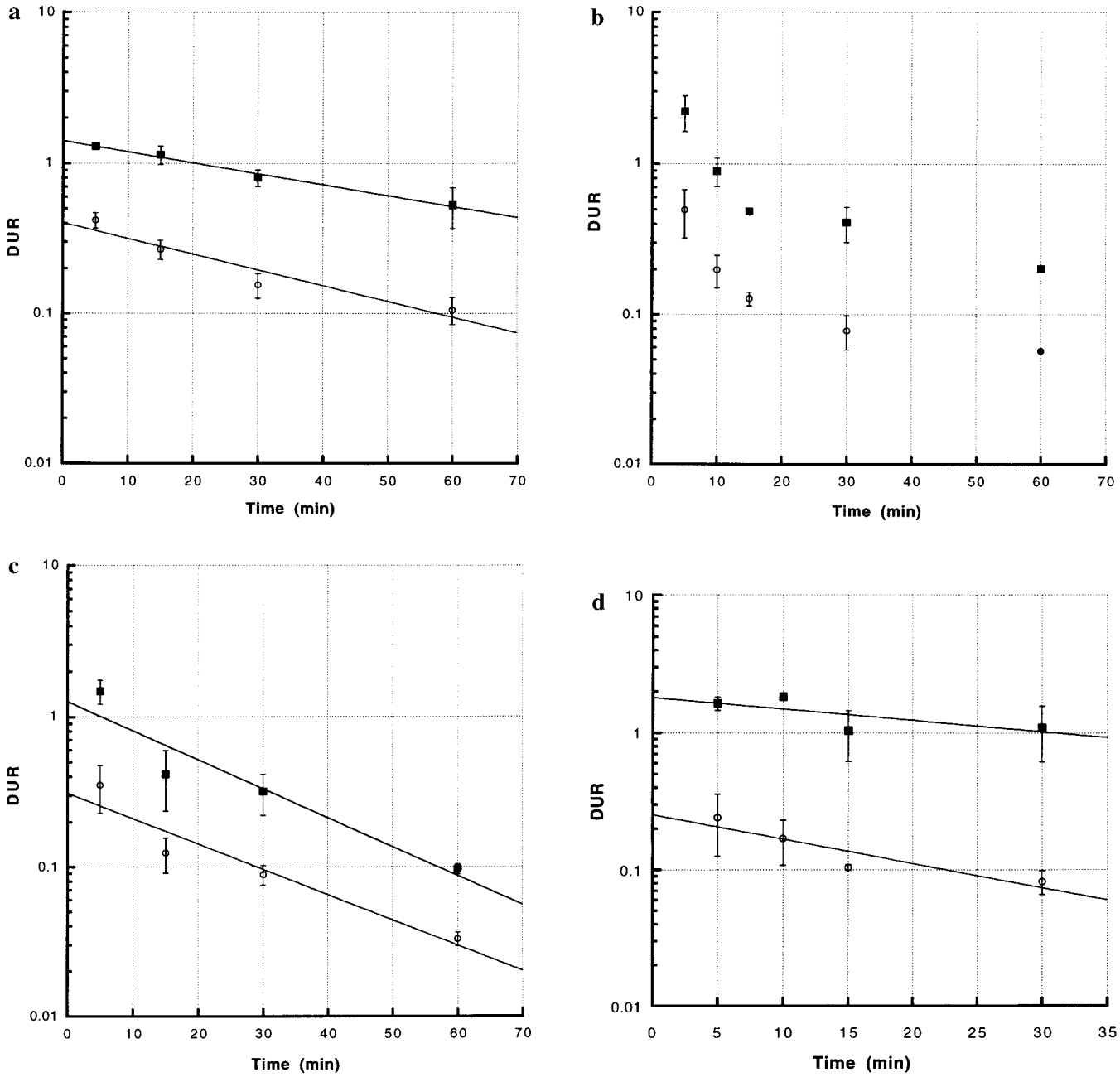


Figure 1. (a) Distribution of [^{11}C]**4a** in rat brain (■) and blood (○) expressed as DUR [(%ID/g) × body weight (g)/100]. The equation for the metabolite-corrected blood is 0.40 $e^{(-0.024*time)}$, and the equation for the brain is 1.41 $e^{(-0.017*time)}$; analyzed with TLC system A ($n = 2$). (b) Distribution of [^{18}F]**4b** in rat brain (■) and blood (○) expressed as DUR. The equation for the metabolite-corrected blood is 0.15 $e^{(-0.017*time)}$, and the equation for the brain is 0.69 $e^{(-0.020*time)}$. This was calculated using the points at 15, 30, and 60 min; analyzed with TLC system A ($n = 4$). (c) Distribution of [^{18}F]**4c** in rat brain (■) and blood (○) expressed as DUR and corrected for metabolites. The equation for the metabolite-corrected blood is 0.31 $e^{(-0.039*time)}$, and the equation for the brain is 1.26 $e^{(-0.044*time)}$; analyzed by TLC using 70% propanol, 30% ammonia as solvent ($n = 4$). (d) Distribution of [^{18}F]**4d** in rat brain (■) and blood (○) expressed as DUR. The equation for the metabolite-corrected blood is 0.25 $e^{(-0.041*time)}$, and the equation for the brain is 1.81 $e^{(-0.019*time)}$. The blood data were also corrected for extraction efficiency; analyzed with TLC system A ($n = 4$).

carboxylic acid moiety or at the *O*-methyl position. The compound appeared to be more lipophilic than the parent. Pike et al. have labeled the desmethyl form of **4a** and found it to have identical binding properties to that of **4a**. However, they did not find the carboxylic acid metabolite.[27] It is not clear if any of these compounds have a superior metabolite profile, i.e., metabolites that are more polar than the parent compound and do not cross the blood−brain barrier.

For receptor-binding ligands that bind reversibly, following a bolus injection, the clearance rate in tissue will eventually match that in blood, and a constant tissue-to-blood concentration ratio will be achieved, a condition termed transient equilibrium. The time to achieve this state depends on the transport and binding kinetics of the tracer. In general, higher-affinity compounds have a slower dissociation rate and will require a longer period of time to achieve transient equilibrium. This relationship can be seen in these data. For the lower affinity compounds, [^{18}F]FBWAY ([^{18}F]**4b**) and Me[^{18}F]FBWAY ([^{18}F]**4c**), the tissue and blood clearance

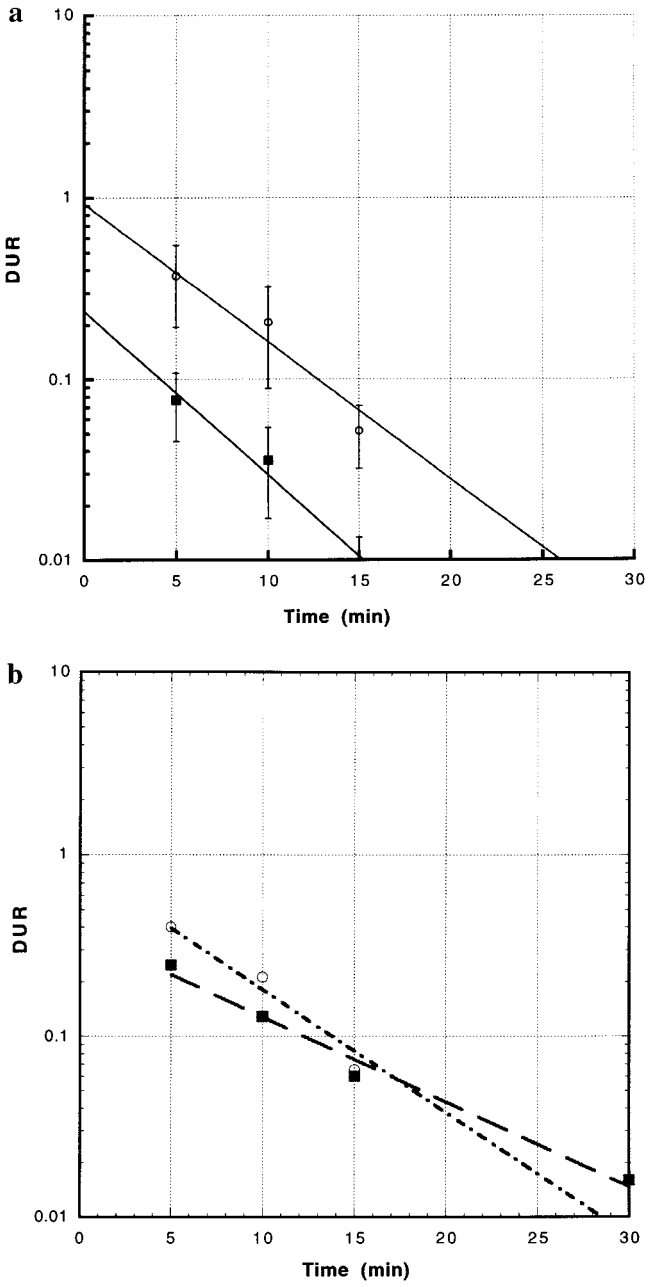


Figure 2. (a) Distribution of [^{18}F]**2c** in rat brain (■) and blood (○) expressed as DUR. The equation for the metabolite-corrected blood is 0.93 $e^{(-0.175*time)}$, and the equation for the metabolite-corrected brain is 0.24 $e^{(-0.208*time)}$; analyzed with TLC system B ($n = 4$). (b) Distribution of [^{18}F]**2d** in rat brain (■) and blood (○) expressed as DUR and corrected for metabolites. The equation for the metabolite-corrected blood is 0.87 $e^{(-0.156*time)}$, and the equation for the metabolite-corrected brain is 0.38 $e^{(-0.108*time)}$; analyzed with TLC system A ($n = 4$). Both brain and blood values were corrected for extraction efficiency assuming that the radioactivity remaining in the pellet was in the form of fluoride.

rates are well-matched (Figure 1b,c) and transient equilibrium was achieved.

However, for the compounds with higher affinity, [^{11}C]-**4a** and [^{18}F]FCWAY ([^{18}F]**4d**), the brain clearance rate was slower than that in blood suggesting that transient equilibrium had not been achieved due to slower equilibrium between bound and free ligand in tissue. For both types of compounds there was a good correlation between the DUR in individual parts of the brain at 30

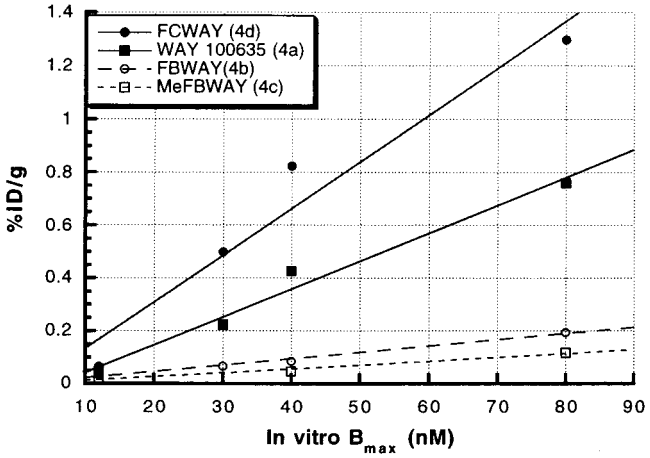


Figure 3. Comparison of the %ID/g in various tissues from rat brain at 30 min after injection with 5-HT_{1A} receptor concentration obtained using quantitative autoradiography in vitro. The rank order of receptor concentration from in vitro studies was hippocampus > frontal cortex > hypothalamus > cerebellum = striatum, and a similar rank order was found in the studies in vivo. The concentration of receptors was taken from Khawaja[27] who determined the receptor concentration from in vitro quantitative autoradiography in rats.

min after injection in rat and the concentration of receptors as determined by in vitro quantitative autoradiography in rats (Figure 3).[28] The rank order of receptor concentration from in vitro studies was hippocampus > frontal cortex > hypothalamus > cerebellum = striatum, and a similar rank order was found in the studies in vivo. The concentration of receptors was taken from Khawaja[27] who determined the receptor concentration from in vitro quantitative autoradiography in rats.

In general, the two cyclohexanecarboxamide analogues ([^{11}C]**4a** and [^{18}F]**4d**) had higher brain-to-blood ratios than the two phenylcarboxamide analogues ([^{18}F]-**4b** and [^{18}F]**4c**) reflecting the in vitro binding affinities and specific binding ratios. Blocking experiments with co-injected 50 nmol of **4a**, reported as %ID/g in the target organ and as a ratio of %ID/g in the ROI minus %ID/g in the cerebellum (Cb) divided by the %ID/g in the Cb (ROI/Cb-1), showed >80% reduction of specific binding in the hippocampus for Me[^{18}F]FBWAY ([^{18}F]-**4c**), whereas [^{18}F]FBWAY ([^{18}F]**4b**) showed only ~60% reduction of specific binding in the hippocampus. The reason for this is not clear given the similar pharmacokinetics, but because of this, Me[^{18}F]FBWAY ([^{18}F]-**4c**) was pursued for evaluation in nonhuman primates.

Other receptors seem not to be involved in brain retention of Me[^{18}F]FBWAY ([^{18}F]**4c**). [^{18}F]**4c** had an affinity of 13 nM for the α_1-adrenoceptor, but co-injection of 200 nmol of prazosin did not decrease the uptake of MeFBWAY (**4c**) in brain. The uptake of prazosin in brain is relatively low (0.06% ID/g) but would still represent a competing dose of ~120 nM in rat brain.[29] MeFBWAY (**4c**) bound to other biogenic amine receptors with affinities > 100 nM.

When blocked with 50 nmol of **4a**, [^{18}F]FCWAY ([^{18}F]-**4d**) showed a decrease in the hippocampus tissue to 54% of the value obtained for the no-carrier-added study (Table 2). The high Ctx/Cb-1 and H/Cb-1 ratios for [^{18}F]-FCWAY ([^{18}F]**4d**) in the presence of 50 nmol of 4a are inconsistent with their similar pharmacokinetics (Fig-

Table 2. Biodistribution (%ID/g) of Radiolabeled WAY Derivatives at 30 min in Rats with and without Co-injected WAY 100635 ($n \geq 4$)

derivative	cortex	hippocampus	hypothalamus	cerebellum	Ctx/Cb-1	H/C6-1
[^{18}F]**4b**	0.085 ± 0.010	0.195 ± 0.049	0.066 ± 0.011	0.034 ± 0.003	1.5	4.7
+50 nmol of **4a**	0.048 ± 0.001	0.074 ± 0.007	0.016 ± 0.041	0.024 ± 0.002	1.0	2.1
[^{18}F]**4c**	0.047 ± 0.007	0.118 ± 0.016	ND	0.020 ± 0.004	1.3	4.9
+50 nmol of **4a**	0.018 ± 0.007	0.017 ± 0.004	ND	0.016 ± 0.003	0.1	0.1
[^{11}C]**4a**	0.426 ± 0.022	0.760 ± 0.118	0.224 ± 0.037	0.045 ± 0.011	8.5	15.9
+50 nmol of **4a**	0.044 ± 0.001	0.132 ± 0.036	0.036 ± 0.012	0.045 ± 0.004	0.0	1.9
[^{18}F]**4d**	0.823 ± 0.028	1.298 ± 0.068	0.499 ± 0.086	0.064 ± 0.006	11.0	19.3
+50 nmol of **4a**	0.267 ± 0.018	0.701 ± 0.053	0.175 ± 0.024	0.048 ± 0.004	4.6	13.6
+200 nmol of **4a**	0.069 ± 0.064	0.085 ± 0.006	0.045 ± 0.009	0.054 ± 0.006	0.3	0.6

ure 1a,d). However, co-injection of 200 nmol of **4a** with [^{18}F]FCWAY ([^{18}F]**4d**), decreased the brain radioactivity to background levels. These data are a single measurement at 30 min and therefore will be dependent on the relative pharmacokinetics of the radiolabeled compound and 50 nmol of **4a**. In addition, **4a** has a higher affinity than either FBWAY (**4b**) and MeFBWAY (**4c**) and therefore should increase the net efflux of these two radiolabeled compounds compared to its effect on [^{11}C]-**4a** and [^{18}F]FCWAY ([^{18}F]**4d**).

Conclusion

We have prepared five F-18-labeled potential 5-HT$_{1A}$ receptor binding ligands. Despite the success in using [^{18}F]fluoromethylbenzoyl derivatives in other applications in vivo, two of these derivatives ([^{18}F]**4e** and [^{18}F]-**4f**) were not readily extracted from either plasma or brain and were abandoned. Of the remaining three, Me-[^{18}F]FBWAY ([^{18}F]**4c**) appears to have an advantage over [^{18}F]FBWAY ([^{18}F]**4b**) because of its higher specific binding in rat as shown by competition studies with **4a**. In vitro test results were consistent with a high-affinity antagonist for the 5-HT$_{1A}$ receptor. [^{18}F]FCWAY ([^{18}F]-**4d**) appears to have the same pharmacokinetic properties as [^{11}C]**4a**. With the longer half-life of F-18, there is the possibility that [^{18}F]**4d** should provide improved imaging qualities and quantification accuracy for 5-HT$_{1A}$ receptor over [^{11}C]**4a**.

Experimental Section

General. Compound **1** was obtained from Med-Life Systems, Inc. Upper Darby, PA. All other chemicals were purchased from Aldrich Chemical Co., Milwaukee, WI. NMR spectra were obtained on a Varian Gemini-2000 (200-MHz) instrument with tetramethylsilane as the internal standard. Mass spectra were recorded on a Hewlett-Packard 5989B mass spectrometer linked to a Hewlett-Packard 5890 series II plus gas chromatograph. Elemental analyses were performed by Galbraith Laboratories (Knoxville, TN). Thin-layer chromatography of the radioactive products was performed on Whatman LK6DF silica gel glass-backed plates (5 × 20 cm, 250 μm). TLC radiochromatograms were obtained using a Bioscan System 200 imaging scanner and a Fuji Bio-imaging Analysis System 1500. Semipreparative HPLC was carried out using a Perkin-Elmer series 200 LC pump. UV absorbance was monitored with a Waters 486 UV detector, and radioactivity was monitored using a Beckman model 170 radioisotope detector. The purification of radiolabeled compounds was performed using a reversed-phase semipreparative Rainin Microsorb 5-μm (10 × 250 mm) C18 column.

***N*-{2-[4-(2-Methoxyphenyl)piperazino]ethyl}-*N*-(2-pyridyl)cyclohexanecarboxamide] (WAY 100635, 4a).** This compound was prepared from cyclohexanecarbonyl chloride and WAY 100634 (**1**) by the procedure of Pike et al.:[18] ^{1}H NMR ($CDCl_3$) δ 0.9−1.3 (m, 4H), 1.4−1.8 (m, 6H), 2.2 (t, 1H), 2.5−2.7 (m, 6H), 3.0 (s, 4H, broad), 3.82 (s, 3H), 3.98 (t, 2H), 6.8−7.0 (m, 4H), 7.2−7.3 (m, 2H),7.74 (td, 1H), 8.51 (dd, 1H); MS (CI-NH_3) *m*/*z* 203 (M + NH_4)$^+$, 186 (M + H)$^+$. Anal. ($C_9H_{15}NO_3$) Calcd: C, 58.38; H, 8.11; N, 7.57. Found: C, 58.67; H, 8.20; N, 7.57.

4-Fluoro-*N*-{2-[4-(2-methoxyphenyl)piperazino]ethyl}-*N*-(2-pyridinyl)benzamide (FBWAY, 4b). This compound was prepared from 4-fluorobenzoyl chloride and **1** by the procedure of Zhuang et al.[14] in 55% yield: mp 260 °C (HCl salt);^{1}H NMR ($CDCl_3$) δ 2.80 (overlapping signals, 10H), 3.84 (s, 3H), 4.30 (t, 2H), 6.90 (m, 8H), 7.39 (m, 3H), 8.44 (dd, 1H); MS (EI) *m*/*z* 434 (M). Anal. ($C_{25}H_{27}N_{14}O_2F\cdot 2HCl\cdot 5H_2O$) Calcd: C, 58.14; H, 5.81; N, 10.85; F, 3.68; Cl, 13.76. Found: C, 58.03; H, 5.95; N, 10.24; F, 3.09; Cl, 12.85.

4-Fluoro-*N*-{2-[4-(2-methoxyphenyl)piperazino]ethyl}-3-methyl-*N*-(2-pyridinyl)benzamide (MeFBWAY, 4c). To a solution of **1** (2.0 g, 6.4 mmol) in 20 mL of methylene chloride were added 1 mL of triethylamine (7.2 mmol) and 1.1 g (6.4 mmol) of 4-fluoro-3-methylbenzoyl chloride (Buu-Hoi and Jacquignon)[30] in 10 mL of methylene chloride, and the mixture was refluxed for 24 h. After the mixture cooled, 100 mL of methylene chloride was added and the solution was placed in a separatory funnel. The solution was treated two times with 100 mL of 1 N $NaHCO_3$ solution and two times with 100 mL of 10% aqueous NaCl. The organic phase was dried with Na_2SO_4. Evaporation of the solvent yielded **4c** as a viscous oil in 92% yield: ^{1}H NMR ($CDCl_3$) δ 2.18 (s, 3H), 2.79 (overlapping signals, 10H), 3.85 (s, 3H), 4.29 (t, 2H), 6.91 (m, 8H), 7.37 (m, 2H), 8.23 (dd, 1H); MS (EI) *m*/*z* 448 (M). Anal. (HCl salt) ($C_{26}H_{29}N_4O_2F\cdot 3HCl\cdot 2H_2O$) Calcd: C, 52.57; H, 6.07; N, 9.44; F, 3.20; Cl, 17.94. Found: C, 52.81; H, 6.31; N, 10.03; F, 2.66; Cl, 17.86.

4-(Fluoromethyl)-*N*-{2-[4-(2-methoxyphenyl)piperazino]ethyl}-3-methyl-*N*-(2-pyridinyl)benzamide (FMeBWAY, 4e). This compound was prepared from 4-(fluoromethyl)-benzoyl chloride and **1** in 60% yield as an oil. The benzoyl chloride was synthesized from thionyl chloride and 4-(fluoromethyl)benzoic acid:[19] ^{1}H NMR ($CDCl_3$) δ 2.80 (overlapping signals, 10H), 3.85 (s, 3H), 4.43 (t, 2H), 5.32 (d, 2H), 7.34 (m. 5H), 8.44 (dd, 1H); MS (EI) *m*/*z* 448 (M). Anal. ($C_{26}H_{29}N_4O_2F\cdot 2H_2O$) Calcd: C, 64.46; H, 6.82; N, 11.57; F, 3.93. Found: C, 64.10; H, 6.17; N, 11.01; F, 3.75.

Compound NFMeBWAY (**4f**) was prepared with a similar procedure as an oil.

Cyclohexanecarbonylglycine (6a). This solid compound was prepared by the procedure used to synthesize 4-fluorobenzoylglycine utilizing cyclohexanecarbonyl chloride: ^{1}H NMR (200 MHz, CD_3CN) δ 1.31 (m, 5H), 1.69 (m, 5H), 2.16 (m, 1H). 3.83 (d, 2H), 6.00 (s, 1H); MS (CI−NH_3) *m*/*z* 203 (M + NH_4)$^+$, 186 (M + H)$^+$. Anal. ($C_9H_{15}NO_3$) Calcd: C, 58.38; H, 8.11; N, 7.57. Found: C, 58.67; H, 8.20; N, 7.57.

4-Fluorobenzoylglycine (6b). This compound was prepared by reacting sodium glycinate in aqueous NaOH with 4-fluorobenzoyl chloride. The 4-fluorobenzoylglycinate was neutralized with HCl. After filtration, a white solid was obtained that was dissolved in acetonitrile. The acetonitrile was evaporated, and chloroform was added to the residue. The mixture was placed onto a silica gel column and eluted with chloroform, which removed 4-fluorobenzoic acid. Then elution with acetonitrile gave 4-fluorobenzoylglycine as a white solid (37%): ^{1}H NMR (CD_3CN) δ 4.05 (d, 2H), 7.22 (m, 2H), 7.38 (s,

1H), 7.87 (m, 2H); MS (CI-NH_3) *m*/*z* 215 $(M + NH_4)^+$, 198 $(M + H)^+$. Anal. ($C_9H_8NO_3F$) Calcd: C, 54.82; H, 4.06; N, 7.11; F, 9.65. Found: C, 54.80; H, 4.12; N, 6.89; F, 9.33.

4-Fluoro-3-methylbenzoylglycine (6c). This solid compound was prepared by the procedure used to synthesize 4-fluorobenzoylglycine utilizing 4-fluoro-3-methylbenzoyl chloride in 14% yield: ^{1}H NMR (CD_3CN) δ 2.31 (s, 3H). 4.05 (d, 2H), 7.14 (t, 1H). 7.35 (s, 1H), 7.70 (m, 2H); MS (CI-NH_3) *m*/*z* 229 $(M + NH_4)^+$, 212 $(M + H)^+$. Anal. ($C_{10}H_{10}NO_3F$) Calcd: C, 56.87; H, 4.74; N, 6.64; F, 9.01. Found: C, 56.69; H, 4.80; N, 6.63; F, 8.66.

Pentamethylbenzyl 4-(*N*,*N*-Dimethylamino)benzoate (7b). To a solution of 4-(*N*,*N*-dimethylamino)benzoic acid (0.57 g, 3.4 mmol) in 15 mL of *N*,*N*-dimethylformamide were added pentamethylbenzyl chloride (PMBC) (0.65 g, 3.3 mmol) and triethylamine (TEA) (0.46 mL, 3.3 mmol). The mixture was stirred at room temperature overnight. The reaction mixture was poured into 80 mL of 10% sodium bicarbonate solution to give white precipitation. The solid product was collected by filtration and dried under vacuum. The crude product was recrystallized from methylene chloride/hexane to give white crystals (0.7 g, 63.6%): mp 154−155 °C; ^{1}H NMR ($CDCl_3$) δ 2.2−2.4 (3s, 15H), 3.01 (s, 6H), 5.42 (s, 2H), 6.60 (d, 2H), 7.90 (d, 2H); MS (EI) 325 (M^+), 160, 148.

Pentamethylbenzyl 4-(*N*,*N*-Dimethylamino)-3-methylbenzoate (7c). To a solution of 4-(*N*,*N*-dimethylamino)-3-methylbenzoic acid (0.70 g, 3.9 mmol) in 15 mL of *N*,*N*-dimethylformamide were added pentamethylbenzyl chloride (0.73 g, 3.7 mmol) and triethylamine (0.52 mL, 3.7 mmol). The mixture was stirred at room temperature overnight. The reaction mixture was poured into a 100-mL 10% sodium bicarbonate solution to give white precipitation. The solid product was collected by filtration and dried under vacuum. The crude product was recrystallized from methylene chloride/hexane to give white crystals (1.0 g, 75.8%): mp 99−101 °C; ^{1}H NMR ($CDCl_3$) δ 2.2−2.4 (3s, 15H), 2.95 (s, 6H), 5.48 (s, 2H), 6.95 (d, 1H), 8.0 (dd, 1H), 8.4 (d, 1H); MS (EI) 339 (M^+), 179, 160, 145, 131.

Pentamethyl 3-Methyl-4-(trimethylammonium trifluoromethanesulfonate)benzoate (5c). To a solution of **7** (255 mg, 0.75 mmol) in 5 mL of anhydrous methylene chloride was added methyl trifluoromethanesulfonate (90 μL, 0.8 mmol), and the solution was stirred at room temperature overnight. At the end of the reaction, the solvent was evaporated under reduced pressure and the residue was recrystallized from methylene chloride/hexane to give 142 mg (37.5%) of white crystals: mp 216 °C dec; ^{1}H NMR ($CDCl_3$) δ 2.1−2.3 (m, 15H), 2.75 (s, 3H), 3.8 (s, 9H), 5.5 (s, 2H), 7.8−8.1 (m, 3H).

Pentamethyl 4-(Trimethylammonium trifluoromethanesulfonate)benzoate (5b). Compound **5b** was prepared by a similar procedure from **8** with a 51.2% yield: mp 219 °C dec; ^{1}H NMR ($CDCl_3$) δ 2.2−2.4 (3s, 15H), 3.75 (s, 9H), 5.52 (s, 2H), 7.85 (d, 2H), 8.2 (d, 2H).

Pentamethylbenzyl 4-(Bromomethyl)benzoate (5e). To a solution of 4-(bromomethyl)benzoic acid (300 mg, 1.0 mmol) and 1,3-dicyclohexylcarbodiimide (DCC) (210 mg, 1.0 mmol) in 4 mL of methylene chloride was added pentamethylbenzyl alcohol (200 mg, 1.1 mmol). The mixture was stirred at room temperature overnight. The solution was filtered through a small silica gel filter (1 mL) and diluted with hexane. The product (160 mg, 30.4%) was obtained from the above solution as a white crystal: mp 166−168 °C; ^{1}H NMR ($CDCl_3$) δ 2.2−2.4 (3s, 15H), 4.48 (s, 1H), 5.49 (s, 2H), 7.42 (d, 2H), 8.10 (d, 2H); MS (EI) 376 (M^+), 295, 197, 160, 145.

Pentamethylbenzyl 4-(Bromomethyl)-3-nitrobenzoate (5f). Compound **5f** was prepared by a similar procedure as above from 4-(bromomethyl)-3-nitrobenzoic acid and pentamethylbenzyl alcohol with a 29% yield: mp 136−137 °C; ^{1}H NMR ($CDCl_3$) δ 2.2−2.4 (m, 15H), 4.8 (s, 1H), 5.55 (s, 2H), 7.62 (d, 1H), 8.22 (dd, 1H), 8.62 (d, 1H).

***cis*-Pentamethylbenzyl 4-Hydroxycyclohexanecarboxylate (9).** To a solution of a mixture of *cis*- and *trans*-4-hydroxycyclohexanecarboxylic acid (4.8 g, 32.4 mmol) (obtained from hydrolysis of the mixture of *cis*- and *trans*-ethyl 4-hydroxycyclohexanecarboxylate) in 50 mL of *N*,*N*-dimethylformamide was added pentamethylbenzyl chloride (6.5 g, 33.1 mmol) and triethylamine (3.3 g, 32.6 mmol). The mixture was stirred at room temperature overnight. The reaction mixture was poured into a 400-mL 10% sodium bicarbonate solution to give a white precipitate. The solid product was collected by filtration and dried under vacuum. The cis and trans mixture was recrystallized from methylene chloride/hexane to 1.5 g (14.8%) of pure cis isomer as white crystals: mp 148−149 °C; ^{1}H NMR ($CDCl_3$) δ 1.6−1.8 (m, 6H), 1.8−2.1 (m, 2H), 2.1−2.3 (m, 15H), 2.3−2.5 (m, 1H), 3.85 (m, 1H), 5.12 (s, 2H); MS (EI) 304 (M^+), 160, 145.

***cis*-Pentamethylbenzyl 4-[(4-Nitrobenzenesulfonyl)-oxy]cyclohexhanecarboxylate (5d).** To a solution of **9** (0.31 g, 0.1 mmol) in 10 mL of methylene chloride were added 4-nitrobenzenesulfonyl chloride (0.25 g, 0.11 mmol) and triethylamine (0.16 mL, 0.11 mmol). The mixture was stirred at room temperature overnight. At the end of the reaction, the solid was filtered and solvent evaporated, and the residue was purified by flash chromatography on silica gel to give 0.25 g (50.1%) of white crystals after recrystallization from methylene chloride/hexane: mp 142 °C dec; ^{1}H NMR ($CDCl_3$) δ 1.4−1.6 (m, 4H), 1.9−2.1 (m, 4H), 2.2−2.3 (m, 15H), 4.55 (m, 1H), 5.22 (s, 2H), 8.1 (d, 2H), 8.4 (d, 2H).

***trans*-Pentamethylbenzyl 4-Fluorocyclohexanecarboxylate (10).** To a solution of **9** (1.8 g, 5.9 mmol) in 100 mL of methylene chloride was added diethylaminosulfur trifluoride (DAST) (0.79 mL, 6.0 mmol). The mixture was stirred at room temperature for 2 h, and the reaction was quenched with water. The methylene chloride was washed with 50 mL of 1 N HCl twice and dried over anhydrous sodium sulfate. The solvent was evaporated, and the residue was chromatographed on silica gel eluting with 1% ethyl acetate in hexane to give 120 mg of product as a white solid (6.7%): mp 110−111 °C; ^{1}H NMR ($CDCl_3$) δ 1.4−1.7 (m, 4H), 1.8−2.2 (m, 4H), 2.2−2.4 (m, 15H), 4.3−4.7 (md, 1H, fluorine coupling), 5.25 (s, 2H); MS (EI) 306 (M^+), 160, 145.

***trans*-4-Fluorocyclohexanecarcoxylic Acid (FC, 2d).** Compound **10** (120 mg, 0.39 mmol) was dissolved in 0.5 mL of TFA and was then diluted with 20 mL of water after 2 min. The white solid formed during dilution was filtered and aqueous layer extracted with 5 × 20 mL of methylene chloride. The organic solvent was combined and dried over anhydrous sodium sulfate. The solvent was evaporated to give 44 mg (77%) of product as a semisolid: ^{1}H NMR ($CDCl_3$) δ 1.4−1.7 (m, 4H), 1.9−2.3 (m, 4H), 2.3−2.5 (m, 1H), 4.4−4.7 (md,1H, fluorine coupling), 10.9 (s, 1H); MS (EI) 146 (M^+) 126, 108, 97.

***trans*-4-Fluoro-*N*-{2-[4-(2-methoxyphenyl)piperazino]-ethyl}-*N*-(2-pyridyl)cyclohexanecarboxamide (FCWAY, 4d).** To a solution of **2d** (44 mg) in 5 mL of 1,2-dichloroethane was added 0.2 mL α,α-dichloromethyl methyl ether. The mixture was refluxed for 2 h until all acid was converted to acid chloride. The solvent and unreacted α,α-dichloromethyl methyl ether were evaporated under reduced pressure, and the residue was redissolved in 5 mL of methylene chloride. The above solution was added to 120 mg of **1** in 5 mL of methylene chloride containing 50 μL of triethylamine. The mixture was refluxed for another 2 h. The solvent was evaporated and residue redissolved in 1 mL of ethyl acetate and chromatographed on silica gel eluting with ethyl acetate containing 0.5% triethylamine. The fractions containing the product were pooled together, and the solvent was evaporated to give 100 mg of product as an oil (75%):^{1}H NMR ($CDCl_3$) δ 1.1−1.4 (m, 2H), 1.6−2.0 (m, 4H), 2.0−2.3 (m,2H), 2.5−2.7 (m, 6H) 3.0 (s, 4H, broad), 3.85 (s, 3H), 3.98 (t, 2H),4.3−4.7 (md, 1H, fluorine coupling), 6.8−7.0 (m, 4H), 7.2−7.4 (m, 4H), 7.8 (td, 1H), 8.55 (dd, 1H); MS (EI) 440 (M^+), 425, 311, 278, 249, 218, 205, 190, 162. Anal. ($C_{25}H_{33}FN_4O_2F$) Calcd: C, 68.16; H, 7.55; N, 12.72. Found: C, 68.02; H, 7.82, N, 12.52.

Synthesis of F-18-Labeled WAY 100635 Analogues. Anhydrous [^{18}F]fluoride ion was prepared from aqueous fluoride produced by irradiating [^{18}O]H_2O. A typical resolubilization procedure is described below.

To a 1-mL V-vial containing 3 μmol of potassium carbonate in 15 μL of water and 6 μmol of Kryptofix-2.2.2 in 30 μL of acetonitrile was added 300−500 μL of activity (30−50 mCi). The water was remove with argon flow three times using anhydrous acetonitrile on a 105 °C heating block. To above test tube containing the anhydrous [^{18}F]fluoride ion was added3 mg of substrate in 0.1 mL of acetonitrile. The vial was sealed and heated on the heating block for 10 min. The reaction mixture was cooled to room temperature and diluted with 1 mL of ether. The ether solution was passed through a small silica gel column (0.5 mL) to a 5-mL V-vial and rinsed with another 1 mL of ether. The solvent was evaporated with argon flow, and the pentamethylbenzyl ester was hydrolyzed with 0.1 mL of trifluoroacetic acid in 2 min. The trifluoroacetic acid was removed with argon flow, and 40 mL of α,α-dichloromethyl methyl ether was added to the reaction vial. The vial was sealed using a cap with Teflon seal, heated at 90 °C for 5 min, and cooled at room temperature for 2 min. The vial cap was opened, and the vial was put back to the heating block for another 3−5 min until the vial became dry. The vial was cooled again, and 5 mg of substrate in 0.2 mL of acetonitrile containing 5 μL of triethylamine was added. The vial was sealed and heated at 90 °C for 5 min. The vial was cooled in water and the reaction mixture diluted with 0.3 mL of HPLC solvent (40−50% acetonitrile in 5 mM phosphate buffer containing triethylamine at a flow rate of 5 mL/min) and injected onto the reversed-phase HPLC column. The fraction containing the product was collected, diluted with 2 volume of water, and passed through a 1-mL C-18 Bond Elut column. The column was washed with 10 mL of water, and the product trapped on the column was eluted off with 0.5 mL of ethanol.

Metabolite Analysis by TLC. Brewster et al.[31] used two silica gel TLC systems to identify cyclohexanecarboxylic acid (CHCA, **2a**) and its metabolites. The first used acetone/light petroleum/acetic acid (20:40:1 by vol), and the second used 1-butanol/acetic acid/water (4:1:1 by vol). They identified hexahydrohippurate, 3,4,5,6-tetrahydrohippurate, hippurate, cyclohexanecarbonyl glucuronide, benzoyl glucuronide, and the parent cyclohexanecarboxylate. In the first TLC system, they reported R_f values of 0.44 for hexahydrohippurate, 0.31 for the hippurate, and 0.1−0.2 for the glucuronide.

In all systems we observed compounds mostly likely to be conjugates analogous to Brewster's work. We prepared **6a**−**6c** and determined their R_f values in the various chromatographic systems. Radioactivity was observed at the same R_f value and is reported as conjugate in the following analysis. Likewise standards of the potential metabolites were also run to determine the R_f values. We used two silica gel-based TLC systems: (A) 1-propanol/ammonia/acetonitrile (50:30:20) where the R_f values were **4a** 0.90, CHCA and conjugate 0.7, **4c** 0.97, MeFB 0.9, conjugate 0.85. **4d** and its metabolites have comparable R_f values to those found for **4a** with fluoride at 0.4. We also used Brewster's first system (system B) [acetone/light petroleum ether/acetic acid (20:40:1)] where the R_f values were **4a** 0.05, CHCA and conjugate at 0.7, **4c** 0.1, MeFB 0.6, MeFB conjugate 0.05. For **4d** in the same system, fluoride, the parent compound, and the conjugates stayed at the origin and FC traveled to 0.65. We also carried out HPLC separations using a semipreparative C18 reversed-phase column eluted with 65−70% methanol in 0.1 M ammonium formate buffer at pH 6.5.

In Vitro Studies. Receptor binding studies were carried out by NovaScreen. In general, brain tissue was used in conjunction with the 5-HT$_{1A}$ subtype-specific tritiated ligand to determine the K_i of the various compounds. In addition, K_i values (nM) were determined against [^{3}H]8-OH-DPAT binding to a cloned cell line containing human 5-HT$_{1A}$ receptors. Receptor binding was also determined by MDS Panlabs (Bothell, WA). The binding assay was carried out with [^{3}H]8-OH-DPAT as the radioligand and cloned cells as the source of receptor with incubation for 60 min at 25 °C. All data are presented as averages ± SEM of at least six determinations unless otherwise noted.

The [^{35}S]GTP-γS saturation binding to cell membranes was also determined. Receptor-linked G protein activation at 5-HT receptors was determined by measuring the stimulation of [^{35}S]-GTP-γS binding. The K_i for the test compound was determined by inhibition studies of this activation.[32]

Rat Biodistribution Studies. Sprague−Dawley male rats, 200−300 g, were injected intravenously while awake. Carbon dioxide was used for euthanasia according to the NIH guidelines for animal use. In the biodistribution studies the rats were injected via the tail vein with 50 μCi of F-18 or 200 μCi of C-11 radioligand or co-injected with 50 μCi of F-18 or 200 μCi of C-11 radioligand and 50 nmol of cold WAY 100635 (**4a**). The rats were sacrificed at 30 min, and the brain was immediately placed in 0.3 M sucrose on ice. The blood and tissues were excised from each animal and weighed. The brain was dissected on ice, and the various brain regions were weighed. The radioactive content of the blood and various tissues was assessed by gamma counting.

Rat Metabolite Studies. The rats were injected intravenously with 100 μCi of the F-18 compounds or 400 μCi of [^{11}C]-**4a** and sacrificed at 5, 10, 15, 30, or 60 min. Blood and brain were removed, weighed, and counted to determine the total activity. After the blood was centrifuged, the serum was removed, mixed with an equal volume of acetonitrile, and centrifuged. The brain was placed in an equal volume of acetonitrile and homogenized for 15−30-s bursts. Following centrifugation, the radioactive content of the supernatant and pellets was determined. The supernatants were applied to TLC plates, developed, and placed on a phosphorimaging plate overnight. The plates were scanned the next day using a Fuji Bio-imaging Analysis System 1500.

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