4-(2′-Methoxyphenyl)-1-[2′-(*N*-2′′-1,3 pyrimidino)-*p*-[^{18}F]fluorobenzamido]ethylpiperazine

[^{18}F]FPWAY

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Chemical name:	4-(2′-Methoxyphenyl)-1-[2′-(*N*-2′′-1,3 pyrimidino)-*p*-[^{18}F]fluorobenzamido]ethylpiperazine	
Abbreviated name:	[^{18}F]FPWAY	
Synonym:	[^{18}F]FBWAY 1,3 N 4-[^{18}F]-Fluoro-*N*-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(2-pyrimidinyl)benzamide	
Agent Category:	Compound	
Target:	5-HT$_{1A}$ receptors	
Target Category:	Receptor-ligand binding	
Method of detection:	Positron emission tomography (PET)	
Source of signal/ contrast:	^{18}F	
Activation:	No	
Studies:	• *In vitro* • Rodents • Non-primate non-rodent mammals • Non-human primates • Humans	Click on the above structure for additional information in PubChem.

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Background

[PubMed]

4-(2′-Methoxyphenyl)-1-[2′-(*N*-2′′-1,3 pyrimidino)-*p*-[^{18}F]fluorobenzamido]ethylpiperazine ([^{18}F]FPWAY) is a radioligand developed for positron emission tomography (PET) imaging of serotonin-1A (5-HT$_{1A}$) receptors in the central nervous system (1, 2). It is a selective 5-HT$_{1A}$ antagonist labeled with ^{18}F, a positron emitter with a physical $t_{½}$ of 109.7 min (3, 4).

The serotonin (5-hydroxytryptamine (5-HT)) neurotransmission system consists mainly of neurons in the brainstem, with nerve tracts extending from these neurons to many areas of the brain and spinal cord. During firing, the neurons release 5-HT, a neurotransmitter that is involved in the modulation of various important physiologic functions and behavior, such as thermoregulation, cardiovascular function, aggressive and sexual behavior, mood, appetite, and the sleep–wake cycle. The effects of 5-HT are mediated by as many as seven classes of receptor populations (5-HT$_1$ to 5-HT$_7$), many of which also contain several subtypes. There are five receptor subtypes within the G protein-coupled 5-HT$_1$ receptor family, with the 5-HT$_{1A}$ subtype located primarily in the limbic forebrain (the hippocampus, entorhinal cortex and septum). 5-HT$_{1A}$ receptors appear to function both as presynaptic (somatodendritic) autoreceptors in the raphe nuclei and as postsynaptic receptors in the terminal fields. This receptor subtype is involved in the modulation of emotion and the function of the hypothalamus, and is implicated in the pathogenesis of anxiety, depression, hallucinogenic behavior, motion sickness, dementia, schizophrenia, and eating disorders. A radioligand that can be used to assess the *in vivo* densities of 5-HT$_{1A}$ receptors and their changes may facilitate investigation of the relationship of these receptors to various neuropsychiatric diseases and aid in the design of novel drugs for their treatment.

Many psychiatric drugs modulate serotonergic transmission or specifically target the 5-HT$_{1A}$ receptors. Various compounds have been radiolabeled for visualization and quantification of these receptors. WAY 100635 was developed as a highly selective, silent antagonist (possessing no intrinsic agonist activity) of 5HT$_{1A}$ receptors at both pre- and postsynaptic sites. WAY 100635 radiolabeled with ^{11}C at the carbonyl position is an effective radioligand but it is rapidly cleared and metabolized. The short $t_{½}$ of ^{11}C also presents some challenges in clinical application of the radiotracer. A radioligand with slower metabolism and labeled with a longer-lived radioisotope would be a better agent for quantitative PET studies. A number of fluorinated derivatives of WAY 100635 have been developed. In 2000, Lang et al. (5) synthesized [^{18}F]FPWAY with labeling at the carboxamide moiety to minimize metabolites that cross the blood-brain barrier (BBB). [^{18}F]FPWAY appears to be an intermediate-affinity antagonist of the 5-HT$_{1A}$ receptors and may be more sensitive to transient changes in endogenous 5-HT$_{1A}$ levels than high-affinity analogs (6). In this WAY 100635 analog, the pyridine ring is replaced by pyrimidine and the cyclohexane ring is replaced by fluorobenzyl. With ^{18}F in the 4 position of the benzamide, [^{18}F]FPWAY is a very different compound from another WAY

100635 analog with a similar abbreviation, [^{18}F]6FPWAY, which was synthesized by Marchais et al. (7). [^{18}F]6FPWAY contains a N-pyridine with ^{18}F in the 6 position of the pyridine moiety.

Synthesis

[PubMed]

Lang et al. (5, 8) first reported the synthesis of FPWAY based on FBWAY (or *p*-MPPF). FPWAY was synthesized as an FBWAY analog (FBWAY 1,3 N) with a 1,3 pyrimidine substituent. FBWAY was prepared by reacting WAY 100634 with the corresponding acid chloride in methylene chloride. Radiolabeling involved labeling the 4-trimethylamino precursor with ^{18}F to produce the radiolabeled 4-fluorobenzoyl chloride. The product was purified by high-performance liquid chromatography (HPLC). The radiochemical yield ranged between 10 and 20%, and the total synthesis time was between 80 and 90 min. The specific activity was 37,000 GBq (1,000 Ci)/mmol at the end of bombardment (EOB).

Ma et al. (9) described a similar synthesis of FPWAY from {2-[4-(2-methoxyphenyl)-1-piperazinyl]-ethyl}-*N*-(2-pyrimidinyl)amine which was prepared from 2-[4-(2-methoxyphenyl)-1-piperazinyl]ethylamine (obtained commercially) by reaction with 2-chloropyrimidine in ethanol and sodium carbonate overnight. The yield was 79%. The final compound was reacted with 4-fluorobenzoyl chloride in triethylamine and 1,2-dichloroethane overnight to give a final yield of 96%. This precursor was reacted with [^{18}F]– Kryptofix in dimethyl sulfoxide and heated in a microwave oven for 2 min. The product was purified by reverse-phase HPLC and a C-18 Sep-Pak to give a final yield of 25-35% EOB and radiochemical purity of 99.0%.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

In vitro affinity of [^{18}F]FPWAY was studied by Lang et al. (5), and the inhibition constant, K_i, was determined to be <1 nM against [^{18}H]8-hydroxy-2-dipropylaminotetralin ([^{3}H]8-OH-DPAT) binding to a cloned cell line containing 5-HT_{1A} receptors. This was relatively weak when compared with other high affinity WAY analogs (MeFWAY and Trans-FCWAY). The antagonist inhibition concentration (IC_{50})was determined to be 340 nM based on the compound's ability to inhibit relaxation induced by 0.3 μM 8-OH-DPAT. On the basis of the compound's ability to relax guinea pig field-stimulated ileum, the authors concluded that [^{18}F]FPWAY showed no agonist activity.

In vitro metabolism studies of FPWAY and [^{18}F]FPWAY were performed by Ma et al. (9) in cryopreserved hepatocytes of rat and human liver tissue. Metabolites were identified by liquid chromatography–tandem mass spectrometry. In rat hepatocytes, aromatic-ring oxidation was the major metabolism pathway for FPWAY, but there was also amide hydrolysis. Amide hydrolysis products were the major metabolites of FPWAY in human hepatocytes, and the metabolic rate appeared to be higher than for other fluorinated

analogs of WAY 100635 (FBWAY and MeFBWAY). The study suggested that the pyrimidyl for pyridyl substitution in FPWAY made the amide more labile to metabolic hydrolysis. In a [^{18}F]FPWAY imaging study using monkey blood, it was shown that pure [^{18}F]FPWAY could be readily extracted by an organic phase (hexane–etyhl acetate 4:1) from its metabolites in a basic aqueous phase (125 mM KCl-NaOH, pH 12.5). This simplified the determination of the input function.

Animal Studies

Rodents

[PubMed]

Lang et al. (5) studied the clearance of [^{18}F]FPWAY from the rat brain by injecting the compound into rats. [^{18}F]FPWAY appeared to have a two-component net efflux curve. Brain distribution and binding saturability were also determined in rats. The differential uptake ratios (DURs, calculated as % of injected dose x animal weight (g)/100) of [^{18}F]FPWAY at 30 min were 0.403 ± 0.056 ($n \geq 4$), 0.918 ± 0.107, and 0.141 ± 0.019 for the cortex, hippocampus, and cerebellum, respectively. When 200 nmol of WAY 100635 was co-injected along with [^{18}F]FPWAY, the DURs decreased to 0.153 ± 0.040, 0.145 ± 0.040, and 0.131 ± 0.033 for the cortex, hippocampus, and cerebellum, respectively. The hippocampus/cerebellum ratios were 5.5 and 0.1 with and without 200 nM WAY 100635, respectively. The kinetic sensitivity (change in DUR per change in receptor concentration) of this compound appeared to be intermediate between FCWAY and FBWAY.

Jagoda et al. (10) conducted autoradiography, biodistribution, and metabolite studies of [^{18}F]FPWAY and other analogs ([^{18}F]FCWAY and [^{18}F]FBWAY) in rats and mice. The DURs (dose = 1850 kBq (50 μCi); $n = 5$) in rats at 30 min after injection for the hippocampus and cerebellum were 0.92 ± 0.11 and 0.14 ± 0.02, respectively. Their (hippocampus DUR/cerebellum DUR) − 1 ratio ((H/Cb) − 1) was 5.5. With the administration of WAY 100635 (200 nmol), the DURs for the hippocampus and cerebellum decreased to 0.14 ± 0.04 and 0.13 ± 0.03, respectively. The specific binding ratios (hippocampus/cerebellum) were 9, 4.6, and 0.1 for the mice of wildtype, heterozygotes, and receptor knockout mice, respectively. This showed that [^{18}F]FPWAY bound specifically to the 5-HT$_{1A}$ receptors and was sensitive to changes in receptor density. Similar differences in localization patterns were observed in [^{18}F]FPWAY autoradiography studies. The results from the blocking studies with WAY 100635 in rat and mouse brains were consistent with the fact that [^{18}F]FPWAY possesses a lower 5HT$_{1A}$ receptor affinity than do high-affinity analogs. [^{18}F]FPWAY regional bindings in the brains of "awake" mice showed no significant changes in response to treatments with paroxetine (a selective serotonin reuptake inhibitor) or fenfluramine (a serotonin transporter substrate and releasing agent). With use of the "chase" paradigm, a consistent pattern of changes of [^{18}F]FPWAY regional brain binding in awake rats treated with fenfluramine or paroxetine was not observed. Urethane-anesthetized rats treated with fenfluramine also showed no significant changes in the specific binding ratios in the

hippocampus. The study suggested that the *in vivo* serotonin system is tightly controlled so that changes in radioligand affinity alone might not produce significant changes in detection sensitivity for changes in endogenous 5-HT.

Other Non-Primate Mammals

[PubMed]

No publication is currently available.

Non-Human Primates

[PubMed]

Carson et al. (11) studied the brain penetration of [^{18}F]fluorobenzoic acid ([^{18}F]FB), a major acid metabolite of [^{18}F]FPWAY, in rhesus monkeys. PET imaging showed that [^{18}F]FB crossed the BBB, but it did not have a significant effect on 5-HT$_{1A}$ receptor quantification of [^{18}F]FPWAY. HPLC analysis of arterial blood samples also showed no metabolites when [^{18}F]FB was injected.

Giovacchini et al. (6) used PET imaging (10-40 min after injection) in rhesus monkeys to assess the response of [^{18}F]FPWAY to dynamic *in vivo* changes in serotonergic neurotransmission caused by systemic administration of paroxetine. A dose of 259-518 MBq (7-14 mCi) of [^{18}F]FPWAY (specific activity was 111 GBq (3 Ci/μmol) at time of administration), TOA) was given with a bolus-plus-infusion technique to achieve constant radioactivity in all brain regions. The distribution of radioactivity in the brain was consistent with the known concentrations of 5-HT$_{1A}$ receptors. The baseline binding potential (BP$_2$) values of the raphe region, anterior cingulated, and frontal lobe were 0.53 ± 0.06, 1.90 ± 0.08, and 0.93 ± 0.08, respectively. Paroxetine administration (5 mg/kg) caused decreases of raphe BP$_2$s ranging from 8 ± 6% during the first 30 min period to 27 ± 10% during the sixth 30-min period (180-240 min). On the other hand, cortical BP$_2$ values increased by 7 ± 3% during the first 30-min period and by 13 ± 5% during the second 30-min period, and then progressively decreased. The study suggested that dynamic changes in 5-HT$_{1A}$ neurotransmission could be quantified by [^{18}F]FPWAY PET imaging.

Human Studies

[PubMed]

No publication is currently available.

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