[^{18}F]3-Fluoro-5-[(pyridin-3-yl)ethynyl]benzonitrile

[^{18}F]F-PEB

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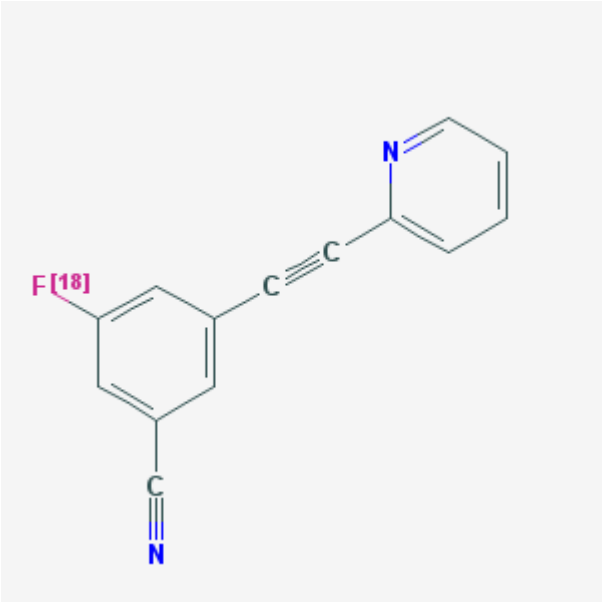
Chemical name:	[^{18}F]3-Fluoro-5-[(pyridin-3-yl)ethynyl]benzonitrile
Abbreviated name:	[^{18}F]F-PEB
Synonym:	
Agent Category:	Compound
Target:	Metabotropic glutamate subtype 5 (mGlu5) receptor
Target Category:	Receptor binding
Method of detection:	Positron Emission Tomography (PET)
Source of signal/ contrast:	^{18}F
Activation:	No
Studies:	• *In vitro* • Rodents • Non-human primates



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Background

[PubMed]

[^{18}F]3-Fluoro-5-[(pyridin-3-yl)ethynyl]benzonitrile ([^{18}F]F-PEB) is a radioligand developed for positron emission tomography (PET) imaging of metabotropic glutamate receptor subtype 5 (mGlu5) in the central nervous system (CNS) (1).

Glutamate is a major excitatory neurotransmitter at CNS synapses. Many neuroanatomical CNS projection pathways contain glutamatergic neurons (2). Glutamate produces its excitatory effects by acting on cell-surface ionotropic glutamate or metabotropic glutamate (mGlu) receptors (3). The mGlu receptors are G-protein-coupled receptors, and the eight mGlu receptor subtypes are further subdivided into groups I, II, and III. The group I receptors include mGlu1 and mGlu5, and they are found mostly in postsynaptic locations. The mGlu5 receptors are found with high to moderate density in the frontal cortex, caudate, putamen, nucleus accumbens, olfactory tubercle, hippocampus, and dorsal horn of the spinal cord, whereas the density in the cerebellum is low. These receptors are coupled to phospholipase C and up- or down-regulate neuronal excitability. They have been implicated in a variety of diseases in the CNS, including anxiety, depression, schizophrenia, Parkinson's disease, and drug addiction or withdrawal. These receptors are also involved in the modulation of various pain states. They thus are attractive targets for therapeutic drug development.

PET and single-photon emission tomography of radioligands targeting mGlu5 receptors can visualize and study the CNS mGlu5 receptors in normal and pathologic states. Some mGlu5 antagonists have been successfully labeled, but their in *vivo* visualization has been hampered by high lipophilicity, unfavorable brain uptake kinetics, or a high metabolism (4, 5). 2-Methyl-6-(phenylethynyl)-pyridine (MPEP) and its methyl analog M-MPEP have been identified as potent and highly selective noncompetitive antagonists for mGlu5. Using the structure of MPEP as a base, Hamill et al. (1) and Patel et al. (5) designed several MPEP analogs as PET radiotracers; [^{18}F]F-PEB was one of the compounds that showed high affinity for mGlu5 receptors with moderate lipophilicity.

Synthesis

[PubMed]

Hamill et al. (1) reported the radiosynthesis of [^{18}F]F-PEB from the aryl chloride via a nucleophilic aromatic substitution reaction. The precursor, 3-chloro-5-[(pyridin-2-yl)ethynyl]benzonitrile, was obtained commercially. In the preparation, Kryptofix222 was added to the aqueous [^{18}F]F– solution in a microwave cavity. The fluoride was dried under argon gas flow by use of microwave pulses (~45 W). The precursor, in dimethyl sulfoxide, was added to the microwave vial. The reaction mixture was then pulsed with the microwave 5 times at 5 s/pulse, with a 30-s pause between pulses. After cooling for 1 min, the reaction was diluted with water and then purified by high-performance liquid chromatography (HPLC). This procedure gave 518 MBq(14 mC) of [^{18}F]F-PEB with a

specific activity of 75.3 ± 9.7 TBq (2,034 ± 359 Ci)/mmol and a radiochemical purity >98%. The time of synthesis was ~ 45 min with a radiochemical yield of 4 ± 0.6% (n = 4) at the end of synthesis (uncorrected).

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Patel et al. (5) used *in vitro* rat brain preparations to determine the *in vitro* mGlu5 binding parameters of unlabeled F-PEB. [^{3}H]MethoxyPyEP, a mGlu5 antagonist, was used in the equilibrium competition studies. The B_{max} for the rat frontal cortex was reported to be 64.7 fmol/mg of tissue (6). The K_i of F-PEB determined from rat cortical membranes was 0.20 ± 0.01 nM (n = 3), and the partition coefficient (log P) was 2.8 (octanol/buffer, pH 7.4). Hamill et al. (1) reported that the 50% inhibitory concentration (IC_{50}; human mGlu5 Ca^{2+} flux assay) was 0.66 ± 0.2 nM (n = 2).

Using a rapid *in vitro* binding assay they had developed, Patel et al. (7) determined the *in vitro* regional distribution of [^{18}F]F-PEB in rat and rhesus monkey brain sections. Rat and rhesus brain sections were incubated with [^{18}F]F-PEB and assayed for 20 min, using a no-wash protocol. The percentages of specific binding ($n \geq 2$) at 20 min were 77 ± 8.1 and 69 ± 22 for rat and rhesus caudate sections, respectively.

In vitro autoradiographic studies (1) of [^{18}F]F-PEB were carried out in rhesus monkey brain slices (from a single monkey). Good specific to nonspecific regioselective labeling with reasonable binding was found in cortex, caudate, putamen, amygdale, hippocampus, and most thalamic nuclei. The cerebellar layers showed less density of binding. In a saturation binding experiment, the B_{max} values for mGlu5 were determined to be 63 and 24 nM in the rhesus caudate and cerebellum, respectively. *In vitro* metabolism of F-PEB was studied by incubation of the radiotracer with human and monkey liver microsomes. Liquid chromatography-tandem mass spectrometry showed that the radiotracer was metabolized slowly by human microsomes. Approximately 70% of the intact compound remained at the end of 30 min. In contrast, the compound was metabolized much faster by monkey liver microsomes: only ~30% of the intact compound remained at the end of 30 min.

Animal Studies

Rodents

[PubMed]

Patel et al. (5) assessed the i*n vivo* distribution of [^{18}F]F-PEB via micro-PET imaging in the rat brain. The rat received 18.5 MBq (0.5 mCi) of [^{18}F]F-PEB (specific activity = 74 TBq (2,000 Ci)/mmol) by intravenous injection. [^{18}F]F-PEB exhibited an excellent *in vivo* specific signal-to-noise ratio. The specific binding and signal-to-noise ratio were reasonably well maintained over 90 min. The corpus striatum/cerebellum ratio was 7.

Other Non-Primate Mammals

[PubMed]

No publication is currently available.

Non-Human Primates

[PubMed]

PET imaging with [^{18}F]F-PEB was carried out in three rhesus monkeys (1). Each monkey received ~185 MBq (5 mCi) of the radiotracer. PET showed rapid brain uptake of [^{18}F]F-PEB radioactivity and substantial mGlu5-specific signals in all gray matter regions, including the cerebellum. The wash-out from the brain was relatively slow and thus produced large, long-lived specific signals. The nonspecific signal was determined from the radioactivity in the white matter with a blocking dose of 3 mg/kg 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine (MTEP), a selective, high-affinity mGlu5 ligand. The total/nonspecific signal ratio at 85 min was 8.1. Coregistered magnetic resonance imaging/PET imaging confirmed that the radioactivity uptake was in the striatum, cortical regions, and cerebellum. The highest concentration of radioactivity was observed in the striatum with ~4.2 standard uptake value (SUV) units at ~55 min. Radioactivity uptake was lower in the frontal cortex and even lower in the cerebellum. These uptakes were blocked by MTEP pretreatment. The distribution of radioactivity seen in PET imaging was consistent with the *in vitro* autoradiography pattern.

Hamill et al. (1) also used HPLC analyses of plasma samples obtained from the monkeys during the above imaging studies to study *in vivo* [^{18}F]F-PEB metabolism. Only one polar metabolite, which could not cross the blood-brain barrier, was observed. The percentages of [^{18}F]F-PEB that remained unchanged were 95.5 ± 1.3, 85.4 ± 3.0, 77.7 ± 3.5, and 62.4 ± 4.5 at 5, 15, 30, and 90 min after administration, respectively.

Human Studies

[PubMed]

No publication is currently available.

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