[18F]-Labeled (R)-(2-(2-(2-methylpyrrolidin-1-yl)ethyl)benzofuran-5-yl)(4-fluorophenyl)-methanone

[18F]9

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Chemical name:	[18F]-Labeled (R)-(2-(2-(2-methylpyrrolidin-1-yl)ethyl)benzofuran-5-yl)(4-fluorophenyl)-methanone
Abbreviated name:	[18F]9
Synonym:	
Agent Category:	Compounds
Target:	Histamine subtype-3 (H₃) receptor
Target Category:	Receptors
Method of detection:	Positron emission tomography (PET)
Source of signal / contrast:	18F
Activation:	No
Studies:	• *In vitro* • Rodents • Non-Human Primates

Structures of compound **12** and [18F]**9** (1).

Background

[PubMed]

[18F]-Labeled (R)-(2-(2-(2-methylpyrrolidin-1-yl)ethyl)benzofuran-5-yl)(4-fluorophenyl)-methanone, abbreviated as [18F]**9**, is a 2-aminoethylbenzofuran-based histamine subtype 3 receptor (H₃R) antagonist/ inverse agonist that was developed for imaging H₃R with positron emission tomography (PET) (1).

H₃R is one of the four G-protein–coupled receptors of the histamine receptor family. The human H₃R gene is located on chromosome 20q13.33, and its products are expressed predominantly in the basal ganglia, hippocampus, and cortical areas, which participate in the synthesis and release of neurotransmitters (e.g., acetylcholine, dopamine, serotonin, and noradrenaline) from histaminergic neurons (2-4). Imbalance of the

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histaminergic interactions results in a number of pathological states, which might be treated with H_3R ligands as neuronal system modulators (5). To date, a large class of H_3R ligands has been reported, which is either imidazole or non-imidazole in structure (1). These ligands have been applied to correct sleep and wakefulness disorders and narcolepsy (6). H_3R antagonists have also been considered to be useful in correcting cognitive disorders and memory processes (7). However, there are many challenges for H_3R ligand development because of the complexity of the central histaminergic system, the diversity of actions, and the overlapping pharmacology of H_3R- and H_4R-targeting compounds (5).

H_3R imaging with specific radioligands has the potential to elucidate changes in the distribution and density of H_3R in living human brain and to determine the dose dependence of the extent and duration of H_3R occupancy by candidates. In general, non-imidazole compounds appear more promising as radiotracers for H_3R imaging. In an effort to develop an ^{18}F-labeled H_3R ligand, Bao et al. selected a new chemotype compound **9**, a non-imidazole 2-aminoethylbenzofuran-based H_3R antagonist/inverse agonist, for imaging feasibility evaluation in animals (1). Compound **9** has previously been shown to have a high affinity and selectivity for human H_3R and to be able to cross the blood–brain barrier (8). In addition, compound **9** can be labeled with no-carrier-added fluorine-18 ($t_{1/2}$ = 109.7 min) at an aryl carbon. The results obtained by Bao et al. have shown that [^{18}F]**9** is an effective radioligand for H_3R imaging in mice and monkeys (1).

Related Resource Links:

Nucleotide and protein sequences of H_3R

Bioassays of H_3R in PubChem BioAssay

Synthesis

[PubMed]

Compound **9** was prepared from commercially available (4-fluorophenyl)(4-hydroxyphenyl)methanone as described previously (1, 8). Bao et al. labeled compound **9** with cyclotron-produced [^{18}F]fluoride ions through its nitro analog precursor **12** ((*R*)-(2-(2-(2-methylpyrrolidin-1-yl)ethyl)benzofuran-5-yl)(4-nitrophenyl)methanone) in a Synthia device equipped with a microwave heater (1). A higher decay-corrected radiochemical yield (RCY, 34%) of [^{18}F]**9** was obtained when the precursor **12** was reacted with [^{18}F]fluoride ions in *N,N*-dimethylformamide than in MeCN (2%) or in dimethyl sulfoxide (6%). The reaction temperature (<90°C) was controlled by setting the microwave power between 50 W and 60 W because decomposition of [^{18}F]**9** became significant above 100°C. [^{18}F]**9** was separated with single-pass reverse-phase high-performance liquid chromatography (HPLC), with a high radiochemical purity (>99%). No residual precursor **12** or other chemical impurities were detected with HPLC in the formulated radioligand. The specific radioactivity of [^{18}F]**9** was 48.51 ± 8.18 GBq/μmol (1,311 ± 221 mCi/μmol; *n* = 12) at the time of intravenous injection to animals (~110 min from the end of radionuclide production). The average RCY of the formulated [^{18}F]**9** was 9.3 ± 5.8% (*n* = 12).

The distribution constant (log $D_{7.4}$) value of [^{18}F]**9** between *n*-octanol and sodium phosphate buffer (0.15 M, pH 7.4) was 2.95 ± 0.06 (*n* = 6). The computed log $D_{7.4}$ (pH 7.4) values of compounds **9** and **12** were 2.90 and 2.45, respectively. The computed partition coefficient (log *P*) values for compounds **9** and **12** were 4.79 ± 0.43 and 4.34 ± 0.34, respectively.

[^{18}F]**9** was found to be 98.8 ± 0.2% intact after 2.5 h in sodium phosphate buffer (pH 7.4) at room temperature (*n* = 6). [^{18}F]**9** was also stable in monkey whole blood (98.4 ± 0.07% intact; *n* = 6) and monkey plasma (99.2 ± 0.1% intact; *n* = 6) for 2.5 h at room temperature.

In Vitro Studies: Testing in Cells and Tissues

Binding of compounds **9** and **12** to a group of human receptors was determined with competitive binding assays (no detailed description of the assays) (1). The assays confirmed the high H_3R affinity of compound **9**, with K_i in the nanomolar range (Table 1). The binding affinities of compound **9** for other human recombinant receptors and binding sites were at least 200-fold lower. The nitro precursor **12** had a binding affinity for human H_3R almost 5-fold higher than that of compound **9** (Table 1).

Table 1. K_i values of compounds 9 and 12 as determined with competitive binding assays.

Receptor or binding site	K_i (nM)	
	Compound **9**	Compound **12**
H_1R	5,440	7,055
H_2R	1,708	923
H_3R	1.9 ± 0.23	0.4 ± 0.04
H_4R	>10,000	>10,000
5-HT$_{1A}$	432	>10,000
5-HT$_{1B}$	>10,000	>10,000
5-HT$_{1D}$	6,380	5,237
5-HT$_{2A}$	2,718	1,355
5-HT$_{2B}$	3,152	>10,000
Dopamine active transporter	>10,000	137
Muscarinic acetylcholine receptor M_1 (M_1)	3,266	931
M_2	462	681
M_3	204	512
M_4	351	415
M_5	437	336
All others*	>10,000	>10,000

*5-HT$_{1E,2C,3,5A,6}$ and 7, $\alpha_{1A,1B,1D,2A,2B}$ and 2C, β_{1-3}, D_{1-5}, $\sigma_{1,2}$, serotonin transporter, and norepinephrine transporter.

Animal Studies

Rodents

Bao et al. first determined the time–activity curve of [^{18}F]**9** in the brain of wild-type FVB mice (1). Images were acquired at baseline ([^{18}F]**9** only; 2.7 ± 1.1 MBq (73 ± 29 µCi)) and after pretreatment (30 min before injection of [^{18}F]**9**) with the selective high-affinity H_3R inverse agonist ciproxifan (2.0 mg/kg), nitro precursor **12** (2.0 mg/kg), or ligand **9** itself (1.0 mg/kg) (n = 3–4 mice/group). Brain uptake of the radioactivity was expressed as standardized uptake value (SUV), where SUV = (% injected dose per cm^3 brain) × (g body weight).

[^{18}F]**9** crossed the blood–brain barrier quickly, with peak whole-brain uptake of 3.36 SUV at ~6.5 min. The brain radioactivity then declined slowly to ~2.3 SUV by 90 min. In mice pretreated with ciproxifan, the brain

radioactivity also quickly peaked, but at a lower level of ~1.98 SUV at 4.5 min after [^{18}F]**9** injection, and then reduced gradually to 1.09 SUV at 90 min. In mice pretreated with nitro precursor **12** or ligand **9**, the [^{18}F]**9** brain radioactivity peaked at 3.5 min, with higher values of 4.34 SUV and 4.07 SUV, respectively. The brain radioactivity then decreased quickly to <1.20 SUV by 90 min. The brain radioactivity uptake was different between the pretreatment experiments with ciproxifan and those with the structural congeners **12** and **9**. One possible explanation is that, at the administered doses, compounds **9** and **12** displace the structurally similar [^{18}F]**9** from plasma proteins, giving a higher plasma free fraction and greater entry of [^{18}F]**9** into brain than in the baseline experiments. Another possibility is that compounds **9** and **12**, but not ciproxifan, blocks [^{18}F]**9** from binding to other unknown sites in the periphery, thereby increasing availability of [^{18}F]**9** for brain entry. Overall, these results indicate that a high proportion of brain radioactivity in the baseline experiments represented specific binding of [^{18}F]**9** to H$_3$R.

Other Non-Primate Mammals

[PubMed]

No references are currently available.

Non-Human Primates

[PubMed]

Bao et al. then determined the time–activity curve of [^{18}F]**9** in the brains of male rhesus monkeys at baseline ([^{18}F]**9** only; 144.67 ± 17.02 MBq (3.91 ± 0.46 mCi); n = 4 monkeys) and after pretreatment with ciproxifan (2.0 mg/kg; n = 3 of the four monkeys) or compound **9** (1.0 mg/kg; n = 2 of the four monkeys) 30 min before [^{18}F]**9** injection (1). PET scans were acquired for 180 min, except for one scan of 120 min. In two of the monkeys, arterial blood was sampled at every 15 s for an initial 2 min, followed by further sampling at 3, 5, 10, 30, 60, 75, 90, 120, 150, and 180 min after [^{18}F]**9** injection. The radioactivity in each region of the brain was expressed as SUV. The two-tissue compartment model was applied to calculate the total distribution volume (V$_T$).

[^{18}F]**9** entered the brain tissue well, with peak uptake in H$_3$R-rich regions, such as striatum (4.45 SUV at ~27.5 min) and frontal cortex (3.86 SUV at ~47.5 min). Peak radioactivity in other regions was lower, but still higher than in cerebellum (2.76 SUV). The radioactivity in all regions decreased slowly to the end of PET scans (180 min). There was no radioactivity uptake into the skull. These results showed that the distribution of [^{18}F]**9** reflected the distribution of H$_3$R. Pretreatment with ciproxifan reduced the peak radioactivity in H$_3$R-rich regions, such as frontal cortex and striatum, but not in cerebellum. Subsequent washout of radioactivity from all H$_3$R-containing regions was slow. Pretreatment with compound **9** reduced peak radioactivity in all brain regions, where the radioactivity declined to a common low level at 180 min. The summed PET images from pretreatment experiments with compound **9** showed a uniform low distribution of radioactivity across brain. Again, there was no radioactivity in the skull. Ciproxifan is known to show species differences in H$_3$R binding affinity, with affinity for human H$_3$R (K_i = 63 nM) >100-fold lower than that for rat H$_3$R (K_i = 0.51 nM). The binding affinity of ciproxifan for monkey H$_3$R is unknown. There is a possibility that the affinity of ciproxifan for monkey H$_3$R is similar to that for human H$_3$R, which may account for incomplete blockade of the brain H$_3$R at the administered dose.

In the baseline experiments, the [^{18}F]**9** radioactivity cleared rapidly from plasma until ~50 min after injection, when the low level of decay-corrected plasma radioactivity became almost constant. Similar plasma time–radioactivity curves were observed in monkeys pretreated with ciproxifan or compound **9**. The plasma free fraction of [^{18}F]**9** was 2.08 ± 0.14% at baseline and 1.4 ± 0.1% in monkeys pretreated with compound **9**.

The [^{18}F]**9** metabolites in the monkey plasma were analyzed after extraction of the radioactivity (95.3 ± 7.44%; n = 115) from plasma with acetonitrile (1). Radio-HPLC showed that the [^{18}F]**9** intact in plasma declined

continuously, while three radiometabolites [^{18}F]**A**–**C** emerged. These radiometabolites appeared to be less lipophilic than [^{18}F]**9** (t_R = 5.63 min), according to their shorter retention times during HPLC. The radiometabolites were also observed in the plasma of monkeys pretreated with ciproxifan. In monkeys pretreated with compound **9**, intact [^{18}F]**9** decreased more quickly to become the minor component, and the least lipophilic radiometabolite [^{18}F]**A** became the major component in plasma. The ability of the radiometabolites to penetrate the blood–brain barrier was unknown. Considering their lower lipophilicities, these radiometabolites may enter brain tissues less readily than [^{18}F]**9** and may be less troublesome for quantification of [^{18}F]**9** binding to H$_3$R. The routes of [^{18}F]**9** metabolism and the identities of the radiometabolites were also unknown. The lack of radioactivity uptake in skull indicated that none of the radiometabolites of [^{18}F]**9** was the [^{18}F]fluoride ion, and that radiodefluorination did not occur for [^{18}F]**9**.

The brain time–activity curves in baseline monkey experiments with [^{18}F]**9** fitted well to both one-tissue and two-tissue (2TC) compartmental models (1). The F test showed that the 2TC model gave the best fit to acquired data (Table 2). On average, ciproxifan reduced the normalized total volume of distribution (V$_T$) 26%–34%, and ligand **9** reduced V$_T$ ~49%–58%, indicating that the majority of the radioactivity in H$_3$R-rich regions of the brain represented specific binding of [^{18}F]**9** to H$_3$R.

Table 2. Estimation of V$_T$ from 2TC model in two monkeys.

Region	Monkey 1 V$_T$			Monkey 2 V$_T$			Mean decrease (%)	
	Baseline	Ciproxifan	**Cold 9**	Baseline	Ciproxifan	**Cold 9**	Ciproxifan	**Cold 9**
Frontal cortex	64.1	43.5	29.5	42.1	26.7	16.4	−34	−58
Striatum	54.7	42.7	29.1	45.3	31.6	19.7	−26	−52
Hippocampus	60.4	45.3	31.2	50.8	30.4	20.8	−33	−54
Thalamus	53.7	40.8	29.9	49.9	29.4	19.4	−33	−53
Cerebellum	42.0	33.5	24.8	28.5	19.6	12.0	−26	−49

Human Studies

[PubMed]

No references are currently available.

References

1. Bao X., Lu S., Liow J.S., Zoghbi S.S., Jenko K.J., Clark D.T., Gladding R.L., Innis R.B., Pike V.W. *Radiosynthesis and evaluation of an (18)F-labeled positron emission tomography (PET) radioligand for brain histamine subtype-3 receptors based on a nonimidazole 2-aminoethylbenzofuran chemotype.* . J Med Chem. 2012;55(5):2406–15. PubMed PMID: 22313227.
2. Leurs R., Bakker R.A., Timmerman H., de Esch I.J. *The histamine H3 receptor: from gene cloning to H3 receptor drugs.* . Nat Rev Drug Discov. 2005;4(2):107–20. PubMed PMID: 15665857.
3. Strakhova M.I., Fox G.B., Carr T.L., Witte D.G., Vortherms T.A., Manelli A.M., Miller T.R., Yao B.B., Brioni J.D., Esbenshade T.A. *Cloning and characterization of the monkey histamine H3 receptor isoforms.* . Eur J Pharmacol. 2008;601(1-3):8–15. PubMed PMID: 18977214.
4. Chepkova A., Yanovsky E., Parmentier R., Ohtsu H., Haas H.L., Lin J.S., Sergeeva O.A. *Histamine receptor expression, hippocampal plasticity and ammonia in histidine decarboxylase knockout mice.* . Cell Mol Neurobiol. 2012;32(1):17–25. PubMed PMID: 21710252.
5. Tiligada E., Kyriakidis K., Chazot P.L., Passani M.B. *Histamine pharmacology and new CNS drug targets.* . CNS Neurosci Ther. 2011;17(6):620–8. PubMed PMID: 22070192.

6. Barbier A.J. andBradbury, M.J. *Histaminergic control of sleep-wake cycles: recent therapeutic advances for sleep and wake disorders.* CNS Neurol Disord Drug Targets. 2007;6(1):31–43. PubMed PMID: 17305552.
7. Esbenshade T.A., Browman K.E., Bitner R.S., Strakhova M., Cowart M.D., Brioni J.D. *The histamine H3 receptor: an attractive target for the treatment of cognitive disorders. .* Br J Pharmacol. 2008;154(6):1166–81. PubMed PMID: 18469850.
8. Cowart M., Faghih R., Curtis M.P., Gfesser G.A., Bennani Y.L., Black L.A., Pan L., Marsh K.C., Sullivan J.P., Esbenshade T.A., Fox G.B., Hancock A.A. *4-(2-[2-(2(R)-methylpyrrolidin-1-yl)ethyl]benzofuran-5-yl)benzonitrile and related 2-aminoethylbenzofuran H3 receptor antagonists potently enhance cognition and attention. .* J Med Chem. 2005;48(1):38–55. PubMed PMID: 15634000.