2-[(4-{[(2-amino-4-oxohydropteridin-7yl)methyl]amino}phenyl) carbonylamino]-4-{*N*-[(4-[^{18}F]-fluorophenyl)carbonylamino] carbamoyl}butanoic acid

[^{18}F]-Folate-1

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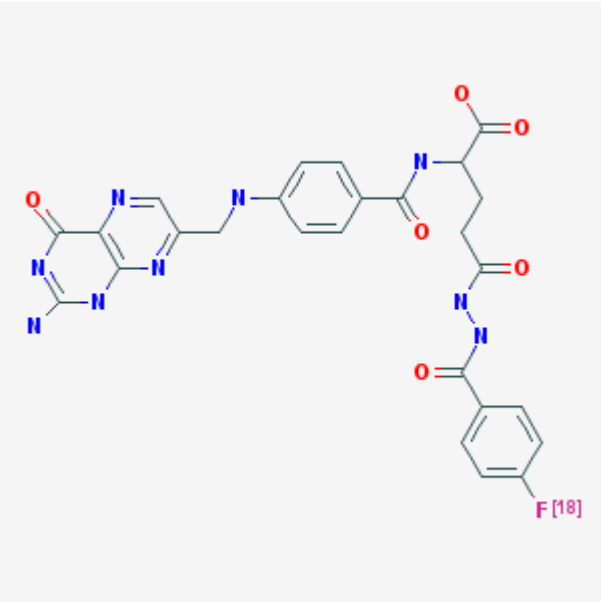
Chemical name:	2-[(4-{[(2-Amino-4-oxohydropteridin-7yl)methyl]amino}phenyl) carbonylamino]-4-{*N*-[(4-[^{18}F]fluorophenyl)carbonylamino] carbamoyl}butanoic acid
Abbreviated name:	[^{18}F]-Folate-1
Synonym:	[^{18}F]-4-fluorobenzenecarbohydrazide-folate; [^{18}F]-1; [^{18}F]-**6**
Agent Category:	Compound
Target:	Folate receptor
Target Category:	Receptor
Method of detection:	Positron emission tomography (PET)
Source of signal / contrast:	^{18}F
Activation:	No
Studies:	• *In vitro* • Rodents



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Background

[PubMed]

Folic acid (FA; also known as folate or vitamin B_9) is a water-soluble vitamin that is required for the synthesis and repair of cellular DNA. FA also acts as a cofactor for many biological reactions and has an important role in cell maintenance and proliferation. Although the folate receptor (FR) is expressed at low levels in normal cells, this receptor is known to be upregulated in cancers such as those of the ovary, lung, breast, brain, colon, and the hematopoietic lineage cells (1). Therefore, the FR system has been targeted with radiolabeled folate and its derivatives, such as ^{67}Ga-deferoxamine (DF)-folate, ^{111}In-diethylenetriamine pentaacetic acid-folate, ^{99m}Tc-mercaptoacetyldiglycine-folate-methotrexate, etc., for the noninvasive detection of malignancies with single-photon emission computed tomography (2). Mathias et al. showed that $^{66/67}$Ga-DF-folate can be used with positron emission tomography (PET) for the imaging of FR-positive cancerous tumors in mice (3). However, although these tracers could detect the FR-rich tumors, they were deemed unsuitable for imaging lesions in the abdominal regions because high levels of radioactivity were observed to accumulate in the liver and intestines of the animals (4). In addition, $^{66/67}$Ga-labeled tracers are known to produce low-resolution images because they generate high positron energy (4.15 MeV and 1.89 MeV for ^{66}Ga and ^{67}Ga, respectively. ^{67}Ga-labeled agents are used for single photon emission computed tomography imaging) compared to ^{18}F (0.64 MeV), which generates superior images and is often used to radiolabel PET imaging agents in the clinic (4). In another study, it was shown that ^{18}F-fluorobenzylamine derivatives of folate could detect tumors that had a high expression of FR, but the radioactivity from these labeled compounds accumulated mainly at the rim of the tumor, and large amounts of the label were retained in the liver and intestines of the animals (4, 5).

In an ongoing effort to generate radiolabeled agents for the imaging of tumors that express high levels of FRs that would be superior to those developed and evaluated earlier, a ^{18}F-fluorobenzene derivative of folic acid ([^{18}F]-folate-1), a pyridinecarbohydrazide-folate derivative of folic acid ([^{18}F]-folate-2), a ^{18}F-fluorobenzene/methotrexate (MTX) conjugate of folic acid ([^{18}F]-folate-MTX-8), and a ^{18}F-pyridinecarbohydrazide-folate/methotrexate conjugate of folic acid ([^{18}F]-folate-MTX-9) have been synthesized (2). The biodistribution of these tracers was investigated in healthy mice. On the basis of results obtained from these studies, [^{18}F]-folate-1 was evaluated for the PET imaging of human KB cell line xenograft tumors (have a high expression of FR) in mice. This chapter describes the results obtained with [^{18}F]-folate-1. Separate chapters in MICAD (http://

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www.micad.nih.gov/) discuss the studies performed with [^{18}F]-folate-2 (6), [^{18}F]-folate-MTX-8 (7), and [^{18}F]-folate-MTX-9 (8).

Related Resource Links

FR-related chapters in MICAD

Adult human FR protein and mRNA sequences

Human FR gene (Gene ID: 2348)

FR clinical trials

FR in Online Mendelian Inheritance in Man (OMIM) database

FR pathway in Kyoto Encyclopedia of Genes and Genomes (KEGG)

Folic acid information on Dailymed site

Synthesis

[PubMed]

Folate-1 was synthesized with hydrazide-folate and 2,5-dioxoazolidinyl 4-fluorobenzoate in presence of triethylamine and the ^{18}F labeling of folate-1 has been detailed elsewhere (9). The total time of synthesis was ~45 min, and the radiochemical yield and radiochemical purity of the final product were >80% (based on the initial ^{18}F concentration) and >97% (without high-performance liquid chromatographic purification), respectively. The specific activity of the final product was reported to be >11.11 MBq/μmol (300 mCi/μmol).

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Using an *n*-octanol-water mixture (pH 7.3), the partition coefficient of [^{18}F]-folate-1 was determined to be 0.38 ± 0.02, indicating that the labeled compound had low lipophilicity (9).

[^{18}F]-Folate-1 was reported to be stable in human plasma for at least 4 h at 37°C (data not presented) (9).

The FR binding affinity of [^{18}F]-folate-1 was determined to be 13.08 ± 0.83 nM with a saturation assay using KB cell membranes (9). A cell internalization assay of [^{18}F]-folate-1 with KB cells (using an acidic buffer, pH not mentioned) showed that 25.85 ± 0.95% of the tracer was internalized by the cells within 20 min at 37°C.

Animal Studies

Rodents

[PubMed]

The biodistribution of [^{18}F]-folate-1 was studied in normal Balb/c mice as described by al Jammaz et al. (2). The animals (n = 4 mice/time point) were injected with 749 kBq (20 μCi) of the tracer through the tail vein and euthanized at 10, 60, and 120 min postinjection (p.i.) to determine the amount of radioactivity accumulated in the various organs. All data were presented as percent of injected dose per gram tissue (% ID/g). A rapid clearance of radioactivity from the blood was observed (4.41 ± 0.60% ID/g at 10 min p.i., 2.55 ± 1.50% ID/g at 60 min p.i., and 1.13 ± 0.51% ID/g at 120 min p.i.). High levels of the label were observed in the kidneys (22.50 ± 4.42% ID/g at 10 min p.i. and 17.88 ± 0.10% ID/g at 120 min p.i.) and the intestine (5.95 ± 2.11% ID/g at 10 min p.i. and 5.86 ± 0.26% ID/g at 120 min p.i.) of these animals. In the liver the amount of label decreased from 7.52 ± 1.43% ID/g at 10 min p.i. to 1.02 ± 0.61% ID/g at 60 min p.i. and to 0.32 ± 0.12% ID/g at 120 min p.i. indicating that the tracer was excreted through the urinary and the hepatobiliary routes. A similar trend of tracer uptake in the various organs was also observed with [^{18}F]-folate-2 (for details, see Chopra (6)).

The biodistribution of [^{18}F]-folate-1 was also investigated in nude mice (n = 4 animals) bearing KB cell tumors. The animals were injected with the radiotracer as described above and euthanized at 60 min p.i (2). The uptake of radioactivity in the various organs of the mice was similar to that observed earlier (see above), and the amount of label in the tumor was 5.94 ± 1.16% ID/g at 60 min p.i. The tumor/blood (T/B) and tumor/muscle (T/M) ratios at 60 min p.i. were 6.39 and 25.83, respectively. The T/B and T/M ratios at 60 min p.i. with [^{18}F]-folate-2 were 14.70 and 28.70, respectively (see Chopra for details (6)). For blocking studies, the mice were injected with 100 μg (0.22 μmol) FA 10 min before the administration of [^{18}F]-folate-1, and the rodents were treated as described above (2). Under these conditions, the uptake of radioactivity in the tumors was reduced significantly (0.74 ± 0.17% ID/g, P <0.05); the kidneys, liver, and intestines (among these organs only the kidneys and the liver have an above normal expression of the FR) also showed a significantly (P < 0.05) reduced accumulation of the label. With [^{18}F]-folate-2, the uptake in the tumor at 60 min p.i. was reduced to 0.66 ± 0.10% ID/g, and the amount of label in the kidneys, liver, and intestines was also reduced significantly (P < 0.05). This indicated that both [^{18}F]-folate-1 and [^{18}F]-folate-2 had a high binding specificity for the FR.

Table: Tumor Uptake and Tumor/Blood and Tumor/Muscle Ratios of different ^{18}F-Labeled Folate

Tracer	Tumor Uptake (% ID/g)	Ratio (No Folate Pre-treatment)

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	No Folate Pre-treatment	With Folate (100 µg) Pre-treatment	Tumor/blood	Tumor/Muscle
[^{18}F]-Folate-1	5.94 ± 1.16	0.74 ± 0.17	6.39	25.83
[^{18}F]-Folate-2	5.74 ± 0.16	0.66 ± 0.10	14.72	28.70

From this study, the investigators concluded that [^{18}F]-folate-2 was probably superior to [^{18}F]-folate-1 for the imaging of FR-rich tumors (2).

Other Non-Primate Mammals

[PubMed]

No publication is currently available.

Non-Human Primates

[PubMed]

No publication is currently available.

Human Studies

[PubMed]

No publication is currently available.

Supplemental Information

[Disclaimers]

No information is currently available.

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