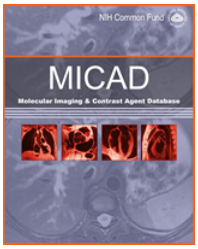
U.S. National Library of Medicine
National Center for Biotechnology Information

NLM Citation: Chopra A. 2-[(4-{[(2-amino-4-oxohydropteridin-7yl)methyl]amino}phenyl) carbonylamino]-4-{*N*-[(2-[18F]-fluoro(4-pyridyl))carbonylamino] carbamoyl}butanoic acid. 2011 Nov 16 [Updated 2011 Dec 26]. In: Molecular Imaging and Contrast Agent Database (MICAD) [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2004-2013.
Bookshelf URL: https://www.ncbi.nlm.nih.gov/books/



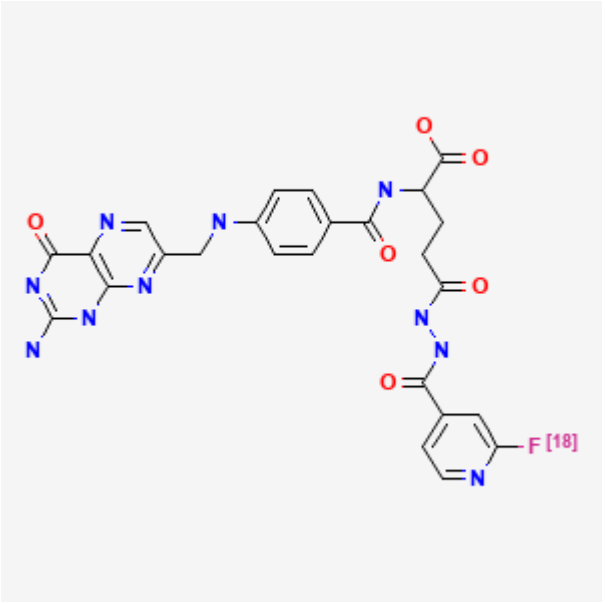
2-[(4-{[(2-amino-4-oxohydropteridin-7yl)methyl]amino}phenyl) carbonylamino]-4-{*N*-[(2-[18F]-fluoro(4-pyridyl))carbonylamino] carbamoyl}butanoic acid

[^{18}F]-Folate-2

Arvind Chopra, PhD[1]

Created: November 16, 2011; Updated: December 26, 2011.

Chemical name:	2-[(4-{[(2-amino-4-oxohydropteridin-7yl)methyl]amino}phenyl) carbonylamino]-4-{*N*-[(2-[^{18}F]-fluoro(4-pyridyl))carbonylamino] carbamoyl}butanoic acid
Abbreviated name:	[^{18}F]-Folate-2
Synonym:	[^{18}F]-2-fluoropyridine-4-carbohydrazide-folate; [^{18}F]-2; [^{18}F]-**9**
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



Click on above structure for information in PubChem.

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 DNA in the cell. 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,

Author Affiliation: 1 National Center for Biotechnology Information, NLM, Bethesda, MD 20894; Email: micad@ncbi.nlm.nih.gov.

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 the intestines of the animals (4). In addition, $^{66/67}$Ga-labeled tracers are known to produce low-resolution images due to their 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 can 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-2 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-2. Separate chapters in MICAD (http://www.micad.nih.gov/) discuss the studies performed with [^{18}F]-folate-1 (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-2 was synthesized with 2,5-dioxoazolidinyl 2-fluoropyridine-4-carboxylate and hydrazide-folate in presence of triethylamine. The ^{18}F labeling of folate-2 is detailed elsewhere (9). The synthesis of [^{18}F]-folate-1 is described in (6). The total time of synthesis for each of the tracers was ~45 min, and the radiochemical yield and radiochemical purity of both the final labeled products were >80% (based on the initial ^{18}F concentration) and >97% (without high-performance liquid chromatographic purification), respectively. The specific activity of each of the radiolabeled compounds 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 coefficients of [18F]-folate-2 and [18F]-folate-1 were determined to be 0.14 ± 0.01 and 0.38 ± 0.02, respectively, indicating that the labeled compounds had a low lipophilicity (9).

Both [18F]-folate-2 and [18F]-folate-1 were reported to be stable in human plasma for at least 4 h at 37°C (data not presented) (2).

The FR binding affinities of [18F]-folate-2 and [18F]-folate-1 were determined to be 15.51 ± 1.80 nM and 13.08 ± 0.83 nM, respectively, with a saturation assay using KB cell membranes (9). A cell internalization assay of [18F]-folate-2 and [18F]-folate-1 with KB cells (using an acidic buffer, pH not mentioned) showed that 11.86 ± 0.43% and 25.85 ± 0.95% of the tracers was respectively internalized by the cells within 20 min at 37°C.

Animal Studies

Rodents

[PubMed]

The biodistribution of [18F]-folate-2 and [18F]-folate-1 was studied in normal Balb/c mice as described by Jammaz et al. (2). The animals (n = 4 mice/time point for each tracer) 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). Radioactivity from [18F]-folate-2 was rapidly cleared from the blood (1.22 ± 0.61% ID/g at 10 min p.i., 0.10 ± 0.05% ID/g at 60 min p.i., and 0.04 ± 0.01% ID/g at 120 min p.i.). High amounts of label accumulated in the kidneys (13.8 ± 5.9% ID/g at 10 min p.i. and 2.92 ± 0.51% ID/g at 120 min p.i.), and low levels of radioactivity were detected in the intestine (0.76 ± 0.20% ID/g at 10 min p.i. and 0.03 ± 0.01% ID/g at 120 min p.i.). This indicated that the tracer was excreted primarily through the urinary route. With [18F]-folate-1, 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 [18F]-folate-1 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, indicating that the tracer was excreted through the urinary and the hepatobiliary routes. The low accumulation of radioactivity with [18F]-folate-2 in the various organs suggested that this tracer had superior pharmacokinetics compared to [18F]-folate-1.

The biodistribution of [18F]-folate-2 and [18F]-folate-1 was also investigated in nude mice (n = 4 animals/tracer) bearing KB cell tumors (2). The animals were injected with the radiotracers as described above and euthanized at 60 min p.i. With both radiolabeled compounds, the uptake of radioactivity in the various organs of the mice at 60 min p.i. was similar to that observed earlier (see above). The amount of tumor accumulation with [18F]-folate-2 and [18F]-folate-1 at 60 min p.i. was 5.74 ± 0.16% ID/g and 5.94 ± 1.16% ID/g, respectively, indicating that with both tracers there was a similar uptake of radioactivity in the tumors. The tumor/blood and tumor/muscle ratios at 60 min p.i. were 14.70 and 28.70, respectively, with [18F]-folate-2 and 6.39 and 25.83, respectively, with [18F]-folate-1. For blocking studies, the mice were injected with 100 μg (0.22 μmol) FA 10 min before the administration of either [18F]-folate-2 or [18F]-folate-1, and the rodents were subsequently treated as before (2). With [18F]-folate-2, the uptake of radioactivity in the tumor at 60 min p.i. was reduced to 0.66 ± 0.10% ID/g ($P < 0.05$), and a similar trend was observed in the kidneys, liver, and intestines ($P < 0.05$); among these organs only the kidneys and the liver show high expression of Fr compared with other normal tissues.

After pretreatment with FA, the uptake of radioactivity from [^{18}F]-folate-1 in the tumors at 60 min p.i. was reduced significantly (0.74 ± 0.17% ID/g, $P < 0.05$); the kidneys, liver and intestines also showed a reduced uptake of the label ($P < 0.05$) at 60 min p.i. This indicated that both [^{18}F]-folate-2 and [^{18}F]-folate-1 had a high binding specificity for the FR in the tumors and the various organs.

From the studies and the results discussed above, the investigators concluded that [^{18}F]-folate-2 was probably superior to [^{18}F]-folate-1 for the imaging of FR-expressing tumors (2). Nude mice (number not reported) bearing KB cell tumors were injected with 11–18.5 MBq (300–500 μCi) [^{18}F]-folate-2 through the tail vein, and whole-body PET images of the animals (under anesthesia) were acquired 45 min p.i. From the images it was clear that radioactivity from [^{18}F]-folate-2 was present primarily in the kidneys, bladder, and the tumor of the animals. No other organ or tissues of the mice were visible in the images. No blocking studies for in vivo imaging were reported.

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

Tracer	Tumor Uptake (% ID/g)		Ratio (No Folate Pre-treatment)	
	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 these studies, the investigators concluded that [^{18}F]-folate-2 was probably a suitable PET imaging agent for the visualization of FR-rich tumors in rodents (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.

References

1. Xia W., Low P.S. *Folate-targeted therapies for cancer.* . J Med Chem. 2010;53(19):6811–24. PubMed PMID: 20666486.
2. Al Jammaz I., Al-Otaibi B., Amer S., Okarvi S.M. *Rapid synthesis and in vitro and in vivo evaluation of folic acid derivatives labeled with fluorine-18 for PET imaging of folate receptor-positive tumors.* . Nucl Med Biol. 2011;38(7):1019–28. PubMed PMID: 21982573.

3. Mathias C.J., Lewis M.R., Reichert D.E., Laforest R., Sharp T.L., Lewis J.S., Yang Z.F., Waters D.J., Snyder P.W., Low P.S., Welch M.J., Green M.A. *Preparation of 66Ga- and 68Ga-labeled Ga(III)-deferoxamine-folate as potential folate-receptor-targeted PET radiopharmaceuticals.* . Nucl Med Biol. 2003;30(7):725–31. PubMed PMID: 14499330.
4. Sega E.I., Low P.S. *Tumor detection using folate receptor-targeted imaging agents.* . Cancer Metastasis Rev. 2008;27(4):655–64. PubMed PMID: 18523731.
5. Bettio A., Honer M., Muller C., Bruhlmeier M., Muller U., Schibli R., Groehn V., Schubiger A.P., Ametamey S.M. *Synthesis and preclinical evaluation of a folic acid derivative labeled with 18F for PET imaging of folate receptor-positive tumors.* . J Nucl Med. 2006;47(7):1153–60. PubMed PMID: 16818950.
6. Chopra, A., *2-[(4-{[(2-amino-4-oxohydropteridin-7yl)methyl]amino}phenyl) carbonylamino]-4-{N-[(4-[18F]-fluorophenyl)carbonylamino] carbamoyl}butanoic acid.* Molecular Imaging and Contrast agent Database (MICAD) [database online]. National Library of Medicine, NCBI, Bethesda, MD, USA. Available from www.micad.nih.gov, 2004 -to current.
7. Chopra, A., *2-[(4-{[(2-amino-4-oxohydropteridin-7yl)methyl]amino}phenyl) carbonylamino]-4-{N-[(2-[18F]-fluoro(4-pyridyl))carbonylamino] carbamoyl}butanoic acid.* Molecular Imaging and Contrast agent Database (MICAD) [database online]. National Library of Medicine, NCBI, Bethesda, MD, USA. Available from www.micad.nih.gov, 2004 -to current.
8. Chopra, A., *2-[18F]Fluoropyridine-4-carbohydrazide-methotrexate (9).* Molecular Imaging and Contrast agent Database (MICAD) [database online]. National Library of Medicine, NCBI, Bethesda, MD, USA. Available from www.micad.nih.gov, 2004 -to current.
9. Jammaz I.A., Otaibi B.A., Okarvi S., Amartey J.K. *Novel synthesis of [18F]-fluorobenzene and pyridinecarbohydrazide-folates as potential PET radiopharmaceuticals.* . Journal of Labelled Compounds and Radiopharmaceuticals. 2006;49(2):125–37.