^{68}Ga-1,4,7-Triazacyclononane,1-glutaric acid-4,7-acetic acid-1,2-diaminoethane-γ-5,8-dideazfolic acid (P3238)

^{68}Ga-P3238

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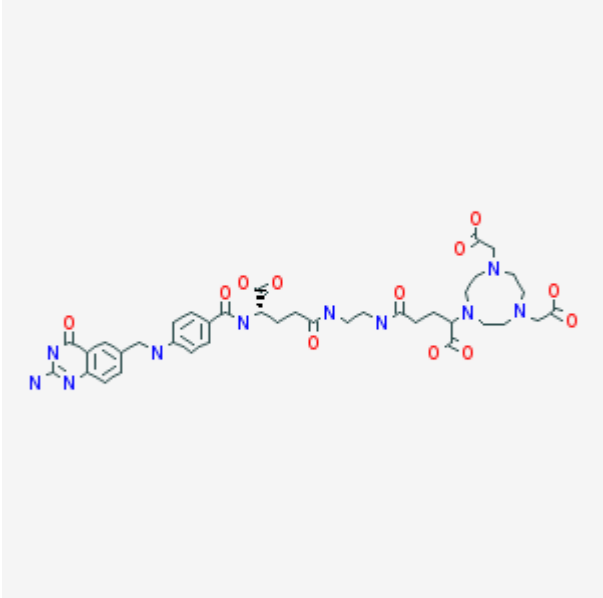
Chemical name:	^{68}Ga-1,4,7-Triazacyclononane, 1-glutaric acid-4,7-acetic acid-1,2-diaminoethane-γ-5,8-dideazfolic acid (P3238)	
Abbreviated name:	^{68}Ga-P3238	
Synonym:	^{68}Ga-NODAGA-5,8-dideazfolic acid	
Agent category:	Compound	
Target:	Folate receptor	
Target category:	Receptor	
Method of detection:	Positron emission tomography (PET)	
Source of signal:	^{68}Ga	
Activation:	No	
Studies:	• *In vitro* • Rodents	Click on the above structure for additional information in PubChem.

Background

[PubMed]

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Folic acid (folate) is a water-soluble B vitamin (1) that is essential for methylation and DNA synthesis. The primary pathway for entry of folate into cells is through the facilitated transporter, which has a low affinity for folate (Michaelis constant (K_m) = 1–5 μM). Some cells in the choroid plexus, kidney, lung, thyroid, spleen, placenta, and thymus also possess a higher-affinity receptor (dissociation constant (K_d) = 0.5 nM) that allows folate uptake *via* receptor-mediated endocytosis. Some human epithelial tumor cells have been found to overexpress folate receptors (2). More than 90% of human ovarian and endometrial cancers express the high-affinity folate receptor, which is absent in the corresponding normal tissues. Breast, colorectal, renal, and lung carcinomas also overexpress the high-affinity folate receptor but at lower frequencies (20%–50%). Activated macrophages, but not resting macrophages, have also been found to have the high-affinity folate receptor (3).

Several folate-based conjugates (^{111}In-DTPA-folate, ^{99m}Tc-EC-folate, and [^{18}F]FBA-folate) have been studied in tumor imaging (4-8). Deferoxamine (DF), a chelating agent, was conjugated to folic acid to form a mixture of two isomers, DF-α-folate and DF-γ-folate. Only the DF-γ-folate isomer was able to displace [^{3}H]folic acid from its receptors, with a 50% inhibition concentration similar to that of folic acid (2.5 nM *versus* 2.4 nM) (9). Fani et al. (10) prepared a γ-folate conjugate with tetraazacyclododecane-*N*,*N*',*N*'',*N*'''-tetraacetic acid (DOTA) and 1,2-diaminoethane as a spacer to form P3026, which was labeled with ^{68}Ga for positron emission tomography (PET) imaging of folate receptors in tumors. To further the quest for a ^{68}Ga-folate conjugate for clinical application, 5,8-dideazfolic acid was conjugated to 1,4,7-triazacyclononane,1-glutaric acid-4,7-acetic acid (NODAGA) *via* 1,2-diaminoethane as a linker between the NODAGA and 5,8-dideazfolic acid (P3238) (11). ^{68}Ga-P3238 was evaluated as a PET agent for imaging folate receptor expression in a mouse tumor model. ^{68}Ga-P3238 exhibited a lower tumor/blood ratio than ^{68}Ga-P3246 (^{68}Ga-NODAGA-folate) but a higher ratio than ^{68}Ga-3026 in the same tumor model.

Related Resource Links:

- Chapters in MICAD (folate receptor)
- Gene information in NCBI (folate receptor)
- Articles in OMIM (folate receptor)
- Clinical trials (folate receptors)
- Drug information in FDA (folate receptor)

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Synthesis

[PubMed]

Fani et al. (11) coupled P3238 (12 nmol) with ^{68}Ga in sodium acetate buffer (pH 4.0) for 10 min at 25°C to yield ^{68}Ga-P3238 with >92% radiochemical purity. Radiochemical yields exceeded 95% with a specific activity of 30 MBq/nmol (0.81 mCi/nmol). ^{67}Ga-P3238 was similarly radiolabeled with a specific activity of ~3 MBq/nmol (0.081 mCi/nmol). ^{68}Ga-P3246 was prepared with similar specific activity as ^{68}Ga-P3238.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

The human nasopharyngeal carcinoma KB cell line folate receptors were studied with ^{67}Ga-P3238 saturation binding studies at 4°C (11). ^{67}Ga-P3238 showed a K_d (affinity constant) of 7.21 ± 2.46 nM, which was slightly higher than the K_d value (5.61 ± 0.96 nM) for ^{67}Ga-P3246. $^{67/68}$Ga-P3238 (2.5 nM) was rapidly associated (bound to the cell surface and internalized) with KB cells at 37°C, with 50% of incubation dose (ID) at 30 min and 60% ID at 4 h. Approximately 10% ID $^{67/68}$Ga-P3238 was internalized at 4 h. Excess folate blocked the cell-associated radioactivity to <1% ID. Approximately 71% of radioactivity was retained in the cells after 4 h incubation in fresh medium.

Animal Studies

Rodents

[PubMed]

Fani et al. (11) performed *ex vivo* biodistribution studies of 0.4 nmol $^{67/68}$Ga-P3238 in nude mice (n = 3–5/group) bearing KB tumor xenografts. Accumulation of $^{67/68}$Ga-P3238 in the KB tumors was 10.95 ± 2.12, 12.89 ± 1.41, and 14.88 ± 2.28% injected dose/gram (ID/g) at 1, 2, and 4 h after injection, respectively. The organ with the highest accumulation at 4 h after injection was the kidneys (112% ID/g), followed by the salivary gland (10.91% ID/g), adrenal (3.8% ID/g), pancreas (2.8% ID/g), liver (2.5% ID/g), heart (2.1% ID/g), muscle (1.7% ID/g), and stomach (1.7% ID/g). The accumulation in the blood was low (0.1% ID/g) at 4 h. The tumor/blood ratios were 57, 99, 254, and 151 at 1, 2, 4, and 24 h, respectively. Pretreatment with excess folate (40 nmol, 5 min before ^{67}Ga-P3238 injection) reduced the radioactivity accumulation by >84% in the folate receptor-positive tumor, salivary glands, and kidneys at 4 h after injection. Pretreatment with pemetrexed (60 min before $^{67/68}$Ga-P3238 injection), a folate analog metabolic inhibitor, significantly reduced the kidney accumulation of ^{67}Ga-P3238 by >70% at 1 h after injection ($P < 0.05$). On the other hand, little inhibition by pemetrexed was observed in the tumor and other organs. $^{67/68}$Ga-P3238 exhibited lower tumor/blood ratios than $^{67/68}$Ga-P3246 (tumor/blood ratios of 81 at 1 h, 207 at 2 h, and 254 at 4 h). No PET imaging study was performed with ^{68}Ga-P3238.

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.

References

1. Stanger O. *Physiology of folic acid in health and disease.* Curr Drug Metab. 2002;3(2): 211–23. PubMed PMID: 12003352.
2. Ke C.Y., Mathias C.J., Green M.A. *The folate receptor as a molecular target for tumor-selective radionuclide delivery.* Nucl Med Biol. 2003;30(8):811–7. PubMed PMID: 14698784.
3. Nakashima-Matsushita N., Homma T., Yu S., Matsuda T., Sunahara N., Nakamura T., Tsukano M., Ratnam M., Matsuyama T. *Selective expression of folate receptor beta and its possible role in methotrexate transport in synovial macrophages from patients with rheumatoid arthritis.* Arthritis Rheum. 1999;42(8):1609–16. PubMed PMID: 10446858.
4. Mathias C.J., Hubers D., Low P.S., Green M.A. *Synthesis of [(99m)Tc]DTPA-folate and its evaluation as a folate-receptor-targeted radiopharmaceutical.* Bioconjug Chem. 2000;11(2):253–7. PubMed PMID: 10725102.
5. 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.
6. Mathias C.J., Wang S., Low P.S., Waters D.J., Green M.A. *Receptor-mediated targeting of 67Ga-deferoxamine-folate to folate-receptor-positive human KB tumor xenografts.* Nucl Med Biol. 1999;26(1):23–5. PubMed PMID: 10096497.
7. Mathias C.J., Wang S., Waters D.J., Turek J.J., Low P.S., Green M.A. *Indium-111-DTPA-folate as a potential folate-receptor-targeted radiopharmaceutical.* J Nucl Med. 1998;39(9):1579–85. PubMed PMID: 9744347.
8. 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):1153–1160. PubMed PMID: 16818950.

9. Wang S., Lee R.J., Mathias C.J., Green M.A., Low P.S. *Synthesis, purification, and tumor cell uptake of 67Ga-deferoxamine--folate, a potential radiopharmaceutical for tumor imaging.* Bioconjug Chem. 1996;7(1):56–62. PubMed PMID: 8741991.
10. Fani M., Wang X., Nicolas G., Medina C., Raynal I., Port M., Maecke H.R. *Development of new folate-based PET radiotracers: preclinical evaluation of Ga-DOTA-folate conjugates.* Eur J Nucl Med Mol Imaging. 2011;38(1):108–19. PubMed PMID: 20799032.
11. Fani M., Tamma M.L., Nicolas G.P., Lasri E., Medina C., Raynal I., Port M., Weber W.A., Maecke H.R. *In Vivo Imaging of Folate Receptor Positive Tumor Xenografts Using Novel (68)Ga-NODAGA-Folate Conjugates.* Mol Pharm. 2012;9(5):1136–45. PubMed PMID: 22497506.