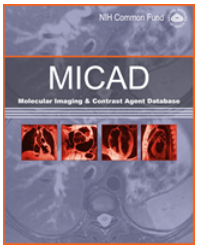
U.S. National Library of Medicine
National Center for Biotechnology Information

NLM Citation: The MICAD Research Team. 2-(2-Nitro-1*H*-imidazol-1-yl)-*N*-(2,2,3,3,3-[^{18}F]pentafluoropropyl)-acetamide. 2005 Nov 8 [Updated 2005 Dec 31]. 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/



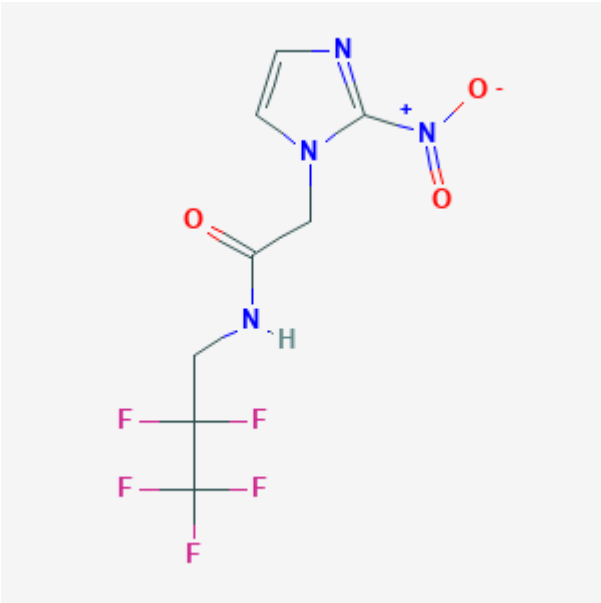
2-(2-Nitro-1*H*-imidazol-1-yl)-*N*-(2,2,3,3,3-[^{18}F]pentafluoropropyl)-acetamide

[^{18}F]EF5

The MICAD Research Team

Created: November 8, 2005; Updated: December 31, 2005.

Chemical name:	2-(2-nitro-1*H*-imidazol-1-yl)-*N*-(2,2,3,3,3-[^{18}F]pentafluoropropyl)-acetamide
Abbreviated name:	[^{18}F]EF5
Synonym:	[^{18}F]-2-(2-nitro-1*H*-imidazol-1-yl)-*N*-(2,2,3,3,3-[^{18}F]pentafluoropropyl)-acetamide
Agent Category:	Compound
Target:	Hypoxic cells
Target Category:	Intracellular reduction and binding
Method of detection:	PET
Source of signal:	^{18}F
Activation:	No
Studies:	• *In vitro* • Rodents



Click on the above structure for additional information in PubChem.

Background

[PubMed]

Hypoxia in malignant tumors is thought to be a major factor limiting the efficacy of chemotherapy and radiotherapy, and its accurate diagnosis is considered a very important and urgent problem to address. This has led to the search for and development of hypoxia-targeted imaging techniques and non-invasive markers of tumor hypoxia. Among those, ^{18}F-labeled nitroimidazoles - used in conjunction with positron emission tomography (PET) – offer a alternative that is less invasive and less prone to sampling errors than the Eppendorf (oxygen) electrode method (1, 2).

Fluoromisonidazole ([^{18}F]FMISO) is the nitroimidazole derivative most widely used with PET. Novel 2-nitroimidazoles, such as ^{18}F-labeled fluoroetanidazole ([^{18}F]FETA) (3), fluoroerythronitroimidazole ([^{18}F]FETNIM) (4), 4-bromo-1-(3-fluoropropyl)-2-nitroimidazole (4-Br[^{18}F]FPN) (5), and 2-(2-nitroimidazol-1*H*-yl)-*N*-(3-fluoropropyl)acetamide ([^{18}F]EF1) (6), are currently under investigation as PET markers for hypoxia.

The pentafluorinated molecule EF5 (2-(2-nitro-1*H*-imidazol-1-yl)-*N*-(2,2,3,3,3-pentafluoropropyl)-acetamide), previously used for measurement of hypoxia by immunohistochemistry (7) and flow cytometry (8), is currently being investigated as a PET agent for hypoxia. [^{18}F]EF5 has the advantage of being more lipophilic than [^{18}F]EF1 (octanol-water partition coefficient of 5.7 for [^{18}F]EF5 *versus* 0.35 for [^{18}F]EF1) because of its multiple fluorine atoms. [^{18}F]EF5 can easily access all tissues, including those in the nervous system.

The oxygen-dependent metabolism of [^{18}F]EF5 is an intracellular process consisting of a series of one-electron reductions. The nitro-radical anion produced in the first reduction step is very reactive toward oxygen, leaving no substrate for the second step of the reduction process. In contrast, a low-oxygen environment induces further reductive reactions that ultimately lead either to the formation of reactive products that can covalently bind to cell components or to charged species that diffuse slowly out of the tissues (1). The reactive products observed during this multistep process include nitroso ($2e^-$), hydroxylamine ($4e^-$), and amine ($6e^-$) derivatives. When fragmentation of the imidazole ring occurs, reactive portions of the molecule, such as glyoxal, bind to macromolecular components of cells in tissues and tumors (2).

Synthesis

[PubMed]

[^{18}F]EF5 can be produced by direct fluorination of the precursor 2-(2-nitro-1*H*-imidazol-1-yl)-*N*-(2,3,3-trifluoroallyl)-acetamide by [^{18}F]F_2 in trifluoroacetic acid, as described by Dolbier et al. (9). The yield obtained by this method is greater than 10%.

Synthesis details have also been reported by Ziemer et al. (10). Their described procedure also involves adding fluorine gas across the double bond of an allyl precursor in trifluoroacetic acid at 0 °C. After evaporation under vacuum, the products are purified by high-performance liquid chromatography (HPLC). After dissolution in physiologic saline, the purified radioactive drug is added to unlabeled EF5. The maximum specific activity for [^{18}F]EF5 synthesis is about 37×10^3 MBq (1,000 mCi)/mmol (when using 7400 MBq (200 mCi) of radioactivity in 70 µmol of fluorine carrier, and assuming a 30% labeling efficiency).

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

No *in vitro* study using the PET agent [^{18}F]EF5 has been reported in the literature to date. Nevertheless, data on the distribution and levels of EF5 binding using fluorescence immunohistochemical techniques have been reported for human head-and-neck and cervical squamous cancer cells (11). In those studies, the maximum rate of *in situ* binding varied by a factor of 6.7 between the lowest (24.8) and the highest (160.3) on an absolute fluorescence scale. Intratumoral heterogeneity was observed for tumors with high binding regions. In contrast, this heterogeneity was minimal for tumors with minimal binding.

Animal Studies

Rodents

[PubMed]

Biodistribution studies using male Fischer rats bearing Morris 7777 (Q7) tumors and 9L glioma were performed by Ziemer et al. (10). The rats were injected with approximately 50-100 µCi of [^{18}F]EF5 and 100 µmol/kg of nonradioactive EF5 (used as added carrier). Images were taken at 10, 60, 120, and 180 min post injection through the femoral vein. [^{18}F]EF5 was shown to be distributed fairly uniformly, with activity uptake increasing over time in the kidneys and gastrointestinal (GI) tract. This increase suggests that [^{18}F]EF5 is excreted via the

urinary system, consistent with prior research studies of EF5 in rodents (12). In contrast, the activity in the liver appeared to be roughly constant and decreasing slowly over time.

At 60 min post injection, the values for the ratio of activity in organs to activity in muscle were as follows: 2.0 (liver), 2.5 (kidney), and 2.7 (GI tract). At 120 min post injection, those ratios were 1.9 (liver), 2.8 (kidney), and 3.9 (GI tract) (as reported in figure 2 of Ziemer et al. (10)). For Q7 tumor -bearing rats, the following tumor/muscle activity ratios (TMR) were obtained: 1.5 (kidney), 1.4 (liver), and 1.3 (GI tract) at 60 min post injection; and 1.2 (kidney), 1.7 (liver), and 1.6 (GI tract) at 120 min post injection (as reported in figure 3 of Ziemer et al. (10)).

Additional measurements of TMR values based on gamma counts led to consistently higher values than those obtained with PET imaging. As an example, the TMR value obtained for a Q7 tumor of size $14 \times 14 \times 14 = 2{,}744$ mm^3 was 1.73 with PET imaging but 2.43 when gamma-counted. This difference was thought to be likely due to the small tumor size and the partial volume effect.

Other Non-Primate Mammals

[PubMed]

No publication is currently available.

Non-Human Primates

[PubMed]

No publication is currently available.

Human Studies

[PubMed]

No publication reporting on [18F]EF5 as a PET agent in human studies is currently available. Nevertheless, EF5 is approved for use in humans and is currently in phase II clinical trials using antibody techniques for the detection of hypoxic cells (13).

References

1. Nunn A, Linder K, Strauss HW. Nitroimidazoles and imaging hypoxia. Eur J Nucl Med. 1995;22(3):265–280. PubMed PMID: 7789400.
2. Hodgkiss RJ. Use of 2-nitroimidazoles as bioreductive markers for tumour hypoxia. Anticancer Drug Des. 1998;13(6):687–702. PubMed PMID: 9755725.
3. Rasey JS, Hofstrand PD, Chin LK, Tewson TJ. Characterization of [18F]fluoroetanidazole, a new radiopharmaceutical for detecting tumor hypoxia. J Nucl Med. 1999;40(6):1072–1079. PubMed PMID: 10452326.
4. Yang DJ, Wallace S, Cherif A, Li C, Gretzer MB, Kim EE, Podoloff DA. Development of F-18-labeled fluoroerythronitroimidazole as a PET agent for imaging tumor hypoxia. Radiology. 1995;194(3):795–800. PubMed PMID: 7862981.
5. Yamamoto F, Aoki M, Furusawa Y, Ando K, Kuwabara Y, Masuda K, Sasaki S, Maeda M. Synthesis and evaluation of 4-bromo-1-(3-[18F]fluoropropyl)-2-nitroimidazole with a low energy LUMO orbital designed as brain hypoxia-targeting imaging agent. Biol Pharm Bull. 2002;25(5):616–621. PubMed PMID: 12033502.
6. Kachur AV, Dolbier WR, Evans SM, Shiue CY, Shiue GG, Skov KA, Baird IR, James BR, Li AR, Roche A, Koch CJ. Synthesis of new hypoxia markers EF1 and [18F]-EF1. Appl Radiat Isot. 1999;51(6):643–650. PubMed PMID: 10581679.

7. Evans SM, Joiner B, Jenkins WT, Laughlin KM, Lord EM, Koch CJ. Identification of hypoxia in cells and tissues of epigastric 9L rat glioma using EF5. Br J Cancer. 1995;72(4):875–882. PubMed PMID: 7547234.
8. Evans SM, Jenkins WT, Joiner B, Lord EM, Koch CJ. 2-Nitroimidazole (EF5) binding predicts radiation resistance in individual 9L s.c. tumors. Cancer Res. 1996;56(2):405–411. PubMed PMID: 8542599.
9. Dolbier WR, Li AR, Koch CJ, Shiue CY, Kachur AV. [18F]-EF5, a marker for PET detection of hypoxia: synthesis of precursor and a new fluorination procedure. Appl Radiat Isot. 2001;54(1):73–80. PubMed PMID: 11144255.
10. Ziemer LS, Evans SM, Kachur AV, Shuman AL, Cardi CA, Jenkins WT, Karp JS, Alavi A, Dolbier WR, Koch CJ. Noninvasive imaging of tumor hypoxia in rats using the 2-nitroimidazole 18F-EF5. Eur J Nucl Med Mol Imaging. 2003;30(2):259–266. PubMed PMID: 12552344.
11. Evans SM, Hahn S, Pook DR, Jenkins WT, Chalian AA, Zhang P, Stevens C, Weber R, Weinstein G, Benjamin I, Mirza N, Morgan M, Rubin S, McKenna WG, Lord EM, Koch CJ. Detection of hypoxia in human squamous cell carcinoma by EF5 binding. Cancer Res. 2000;60(7):2018–2024. PubMed PMID: 10766193.
12. Laughlin KM, Evans SM, Jenkins WT, Tracy M, Chan CY, Lord EM, Koch CJ. Biodistribution of the nitroimidazole EF5 (2-[2-nitro-1H-imidazol-1-yl]-N-(2,2,3,3,3-pentafluoropropyl) acetamide) in mice bearing subcutaneous EMT6 tumors. J Pharmacol Exp Ther. 1996;277(2):1049–1057. PubMed PMID: 8627516.
13. Koch CJ, Hahn SM, Rockwell K, Covey JM, McKenna WG, Evans SM. Pharmacokinetics of EF5 [2-(2-nitro-1-H-imidazol-1-yl)-N-(2,2,3,3,3-pentafluoropropyl) acetamide] in human patients: implications for hypoxia measurements in vivo by 2-nitroimidazoles. Cancer Chemother Pharmacol. 2001;48(3):177–187. PubMed PMID: 11592338.