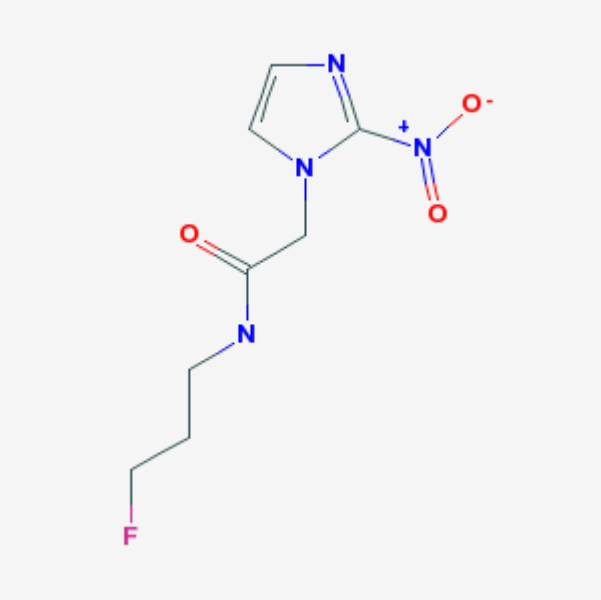
2-(2-Nitroimidazol-1*H*-yl)-(3-[^{18}F]fluoropropyl)acetamide

[^{18}F]EF1

The MICAD Research Team

Created: November 5, 2005; Updated: November 17, 2005.

Chemical name:	2-(2-Nitroimidazol-1*H*-yl)-*N*-(3-[^{18}F]fluoropropyl)acetamide
Abbreviated name:	[^{18}F]EF1
Synonym:	[^{18}F]-2-(2-Nitro-1*H*-imidazol-1-yl)-*N*-(3-fluoropropyl)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

NLM Citation: The MICAD Research Team. 2-(2-Nitroimidazol-1*H*-yl)-(3-[^{18}F]fluoropropyl)acetamide. 2005 Nov 5 [Updated 2005 Nov 17]. In: Molecular Imaging and Contrast Agent Database (MICAD) [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2004-2013.

and urgent problem to address. This has led to the search and development of hypoxia-targeted, non-invasive markers of tumor hypoxia. Among those markers, [^{18}F]-labeled nitroimidazoles, used in conjunction with positron emission tomography (PET), offer an alternative that is less invasive and less prone to sampling error than the Eppendorf (oxygen) electrode method (1, 2).

Fluoromisonidazole ([^{18}F]FMISO) is the most widely used nitroimidazole derivate used with PET. Novel 2-nitroimidazoles, such as [^{18}F]FETA (3), [^{18}F]FETNIM (4), 4-Br[^{18}F]FPN (5), [^{18}F]EF1, and [^{18}F]EF5 (6), are currently being investigated as potential markers of tumor hypoxia.

[^{18}F]EF1 is a molecule comparable to [^{18}F]FETA, with the exception that [^{18}F]EF1 carries an additional carbon on the side chain. [^{18}F]EF1 is also a 3-monofluorinated analog of the pentafluorinated molecule EF5 (2-(2-nitro-1*H*-imidazol-1-yl)-*N*-(2,2,3,3,3-[^{18}F]pentafluoropropyl)acetamide), which is used for the measurement of hypoxia in immunohistochemistry (7) and flow cytometry (8).

The oxygen-dependent metabolism of nitroimidazoles 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 to the formation of either 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 multi-step process include nitroso (2e-), hydroxylamine (4e-), and amine (6e-) derivatives. When the 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]

Two modes of synthesis for [^{18}F]EF1 were reported in 1999 by Kachur et al. (9).

In the first mode, EF1 is synthesized from EBr1 by a nucleophilic substitution of bromine by fluorine using potassium Kryptofix 222 fluoride in dimethyl sulfoxide (DMSO). EBr1 is prepared from 2-(2-nitroimidazol-1*H*-yl)-acetic acid and 3-bromopropylamine hydrobromide using the mixed anhydride reaction with isobutyl chloroformate (10). After dissolution in acetonitrile (MeCN) and storage over molecular sieves, the mixture is cooled to 0°C under argon and stirred with an addition of isobutylchloroformate. A solution of 3-bromopropylamine hydrobromide (219 mg, 1 mmol) and 140 µl of NEt_3 (1 mmol) in 5 ml of MeCN is then added. After stirring, cooling to 0°C, and centrifugation, the resulting precipitate of NEt_3H^+Cl- is extracted and dried at room temperature. The final product, EBr1, is then recrystallized from hexane/EtOAc (10:1 ratio) and dried over molecular sieves. EBr1 is obtained with a yield of approximately 70%.

In the second mode, EF1 is prepared by following the protocol used for EBr1 described above, except that an equivalent quantity of 3-fluoro- instead of 3-bromopropylamine is used. In this case, the C–F bond in EF1 (which is not hydrolyzed) reacts with an excess of triethylamine (unlike the C–Br bond in EBr1). In this second method, the reaction conditions and separation procedure are different: a 2-fold excess of triethylamine is used in the reaction, and the dried reaction mixture, after centrifugation, is dissolved in dry EtOAc. After evaporation and dissolution of the residue in $CHCl_3$, EF1 is extracted by H_2O, and the aqueous layers are then combined and lyophilized. The yield is approximately 15% pure EF1.

The preparation of the radioactive carrier is performed by using [^{18}F]fluoride, obtained by the ^{18}O(p,n) ^{18}F reaction using ^{18}O-enriched water. Details for this preparation are reported by Kachur et al. (9).

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

No publication is currently available.

Animal Studies

Rodents

[PubMed]

Evans et al. (11) showed the ability of [^{18}F]EF1 to differentiate hypoxic *versus* aerobic tumors in rodents, using the hypoxic Morris 7777 (Q7) hepatoma tumor type and the oxic 9LF glioma tumor model. The experimental procedure involved injecting intravenously approximately 1.9 MBq (0.05 mCi) of [^{18}F]EF1 (1% of the animal's weight), 3 mM of the carrier EF1,and 7 mM EF3 (2-(2-nitro-1*H*-imidazol-1-yl)-*N*-(2,2,3,3,3-[^{18}F]trifluoropropyl)acetamide). The specific activity of [^{18}F]EF1 for injection was 5.6×10^4 MBq/mol (1.51×10^3 mCi/mol).

The use of an excess of the carrier drug EF1 and EF3 made it possible to make an independent assessment of the drug biodistribution by more conventional means (e.g., high-performance liquid chromatography (HPLC)), as well as making an immunohistochemical detection of intracellular EF1/EF3 adducts in the tumors using EF3-specific antibodies. Biodistribution of [^{18}F]EF1 was also performed in non-tumor-bearing mice, as a reference. After positron emission tomography (PET) studies, the tissues for all animals were removed and analyzed by γ-counting at 1, 5, 90, and 120 min after injection.

Results showed that for both rat tumor types, low retention of [^{18}F]EF1 was obtained in muscle, heart, spleen and brain, whereas significantly higher levels were found in tumor and bone (tibia). γ-counts of excised organs and tissues were measured at 90 min after injection, and their uptake was expressed as a percentage of injected activity per gram of

sample (% ID/g). The following results (% ID/g) were found for muscle, heart, spleen, brain, tumor, and bone (tibia), respectively (all values are x 10–^{3}): for Q7 tumors, 2.5, 1.5, 3.0, 1.5, 6.5, and 13; for 9LF tumors, 1.5, 1.0, 1.8, 0.6, 2.5, and 12 (11).

Studies using normal mice showed a rapid decrease of activity in all organs except bone (tibia), likely reflecting a bone contamination by [^{18}F]F^{-} and a very substantial increase of urine activity by 90 min after injection (0.6 fraction ID/g). For Q7 tumors, the tumor-to-muscle ratio (T/M) initially decreased (from approximately 2.0 at 10 min after injection to 1.7 at 50 min) and then increased (to approximately 2.4) to reach a plateau 125 min after injection. In contrast, the T/M ratio for 9L tumors never exceeded 1.0, and a steady decrease was observed over time (11).

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. 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. 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.
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. 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.
10. Krejcarek GE, Tucker KL. Covalent attachment of chelating groups to macromolecules. Biochem Biophys Res Commun. 1977;77(2):581–585. PubMed PMID: 409400.
11. Evans SM, Kachur AV, Shiue CY, Hustinx R, Jenkins WT, Shive GG, Karp JS, Alavi A, Lord EM, Dolbier WR, Koch CJ. Noninvasive detection of tumor hypoxia using the 2-nitroimidazole [18F]EF1. J Nucl Med. 2000;41(2):327–336. PubMed PMID: 10688119.