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Design of silicon-based misonidazole analogues and ^{18}F-radiolabelling

Pierre Bohn[a], Abdallah Deyine[a,b], Rabah Azzouz[b], Laetitia Bailly[b], Catherine Fiol-Petit[b], Laurent Bischoff[b,*], Corinne Fruit[b,*], Francis Marsais[b], Pierre Vera[a,*]

[a]*Department of Nuclear Medicine, Henri Becquerel Center and Rouen University Hospital and QuantIF- LITIS (EA4108), 1 rue d'Amiens, 76038 Rouen Cedex, France*

[b]*UMR 6014, COBRA, CNRS, INSA of Rouen and University of Rouen, Place Emile Blondel, B.P. 08, 76130 Mont-Saint-Aignan, France*

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Abstract

Introduction: Development of new ^{18}F-labeled tracers for positron emission tomography (PET) imaging is increasingly important. Herein, we described the synthesis of silicon analogues of [^{18}F]fluoromisonidazole in order to develop new radiolabelled compounds for the detection of tumour hypoxic domain. Their stabilities and their in vivo biodistribution were evaluated.

Methods: ^{18}F-labeled silicon-based misonidazole analogues were synthesized by alkylating 2-nitroimidazole with alkyloxy-(3-chloropropyl) dialkyl or diarylsilane. These intermediates were labeled with [^{18}F]F$^-$ with a mixture of K^{18}F and Kryptofix (K222) in acetonitrile as standard condition. PET imaging was performed using a dedicated small animal PET scanner.

Results: ^{18}F-labeled silicon-based misonidazole analogues were easily synthesized in three steps. The hydrolytic and radiolytic stability of these new fluorosilanes depend on the steric hindrance at the silicon center. Indeed, partial uptake of dimethylfluorosilane [^{18}F]**2a**(1-(3-(Fluorodimethylsilyl)propyl)-2-nitro-1H-imidazole) in tumor hypoxic area was observed but defluorination also appeared. Moreover, PET studies indicated that, owing to its high lipophilicity, the most stable dinaphtylfluorosilane [^{18}F]**2d** is retained mainly by the lungs.

Conclusion: We have described an efficient and versatile approach for the synthesis of ^{18}F-labeled, silicon-based misonidazole analogues. PET imaging of one of these compounds revealed that hypoxia could be detected. Controlling the biodistribution of ^{18}F-labeled silicon-based misonidazole analogues will require additional studies.

Keywords: Fluorine-18; misonidazole; radiolabelling; PET imaging; fluorosilane; hypoxia

1. Introduction

The recent development of positron emission tomography (PET-scan) imaging [1–6] has been correlated with a crucial need for new techniques to introduce radionuclides onto biologically active molecules. Some metals such as gallium have been used as complexes themselves linked to the target molecules. Another widespread strategy consists in establishing a covalent bond between a carbon atom and the radionuclide itself. In this way, the very stable C-F bond allows the incorporation of fluorine-18 in radiotracers [7–11].

Fluorine-18 emits a relatively low-energy positron which annihilates with an electron, emitting two photons of 511 keV. Detection of the latter at 180° by means of PET-scan cameras affords accurate imaging of biological tissues. Two major molecules have emerged, such as 2-deoxy-2-[^{18}F]fluoro-D-glucose (FDG) and [^{18}F]fluorodopa [12,13]. Some other examples were published for various applications, such as 1-α-D-(5-fluoro-5-deoxyarabinofuranosyl)-2-nitroimidazole ([^{18}F]fluoroazomycin arabinoside) and 1-(2-nitroimidazolyl)-3-[^{18}F]fluoro-2-hydroxypropanol ([^{18}F]fluoromisonidazole; FMISO) as hypoxia tracers [14–16].

* Corresponding authors. Corinne Fruit is to be contacted at UMR 6014, COBRA, CNRS, INSA of Rouen and University of Rouen, Place Emile Blondel, B.P. 08, 76130 Mont-Saint-Aignan, France. Tel.: +33 235522482. Pr Laurent Bischoff, UMR 6014, COBRA, CNRS, INSA of Rouen and University of Rouen, Place Emile Blondel, B.P. 08, 76130 Mont-Saint-Aignan, France. Tel.: +33 235522903; fax: +33 235522962, Pr Pierre Vera, Department of Nuclear Medicine, Henri Becquerel Center and Rouen University Hospital and QuantIF- LITIS (EA4108), 1 rue d'Amiens, 76038 Rouen Cedex, France. Tel.: +33 232082255; fax: +33 232082550.

E-mail addresses: laurent.bischoff@univ-rouen.fr (L. Bischoff), corinne.fruit@insa-rouen.fr (C. Fruit), piever@rouen.fnclcc.fr (P. Vera).

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However, the use of C-^{18}F bond-bearing molecules is limited by the low reactivity of fluoride anion in water, which dramatically decreases the reaction rates especially in dilute solutions. Thus, the experimental conditions employed for the creation of this bond, such as nucleophilic substitution of a leaving group (mesylate, tosylate or nitro), are generally drastic. Actually, high temperatures and cation-complexing agents such as (2,2,2) Kryptofix are required to increase the reactivity of fluoride. An efficient breakthrough in this field was accomplished by Gouverneur who used electrophilic fluorine [17,18]. As ^{18}F has a half-life of 110 min, the need for rapid incorporation of fluoride at high dilution is still of huge interest.

An alternative technique consists in creating a Si-F bond instead of C-F bond [19]. In fact, the thermodynamic stability of Si-F bond is known for a long time and used as a very common and helpful method for the cleavage of silyl ethers. Fluorine has a higher bonding energy with silicon (565 kJ.mol^{-1}) than with carbon (485 kJ.mol^{-1}). Despite this high energy bonding, fluorosilanes are moderately stable in water, especially at low concentrations, presumably due to the high propensity of silicon to form stable siloxanes. In vivo observation of fluorosilanes proposed by Rosenthal [20] showed a rapid uptake of ^{18}F in bones. More recently, Schirrmacher and Ametamey [21–26] independently reported efficient labelling based upon the Si–^{18}F bond formation. The first paper appeared in 2006, based on a Si–^{19}F/Si–^{18}F exchange with biomolecules already bearing a ^{19}F trialkyl or alkyldiaryl fluorosilane as a ^{18}F acceptor [21]. This work confirmed the low in vivo stability of fluorosilanes and overcame this drawback by the use of more bulky and stable di*tert*-butylphenylfluorosilanes [22,23]. Most recently, Ametamey's group focused on the fluorolysis of carbon-silicon ethers for the formation of the Si–^{18}F bond using potassium fluoride in the presence of (2,2,2)Kryptofix and acetic acid [24–27]. Indeed, none of both methods permitted the direct radiolabelling with aqueous fluoride. High solvatation of electronegative fluoride ion in water lowers its reactivity, making aqueous fluorination almost impossible under high dilution conditions. In 2005, fluorination of trialkoxysilanes was carried out with hydrofluoric acid at a submillimolar concentration, and scintillography was provided [28]. Moreover, hydrolysis of fluorosilanes could be avoided with bulky groups on the silicon atom.

In this work, we wish to describe the synthesis of new radiolabelled fluorosilanes and to discuss their stability towards hydrolysis and radiolysis. Our target molecule consists of a tracer used for the monitoring of hypoxia. In this context, we turned our attention on the synthesis of silicon analogues of FMISO **1** (Fig. 1). [^{18}F]FMISO is an increasingly attractive radiolabelled 2-nitroimidazole for the detection of tumour hypoxic domain [29,30]. Measurement of oxygen levels in human tumours is crucial for diagnosis and treatment of patients, especially in the development of new therapeutic strategies including radiotherapy [31,32]. Hypoxia in solid tumours induces an aggressive phenotype, promoting tumour progression, increasing metastatic potential and, consequently, limiting the bioavailability of chemotherapeutic agents [33,34]. Nitroimidazoles are known to be involved in metabolic processes which appear with low oxygen concentration in hypoxic tissues. Since the accumulation of [^{18}F]FMISO was shown to be inversely correlated to tissue oxygenation, the severity of hypoxia was reflected by [^{18}F]FMISO concentration. Despite a recent improvement of experimental method [35], synthetic approaches to FMISO still have limitations such as low radiochemical yields [36]. Indeed, low concentrations within hypoxic tissues require longer time experiments for PET-scan imaging. In order to develop new markers for hypoxia, the synthesis of silicon-analogues of this molecule is envisioned as described in Scheme 1.

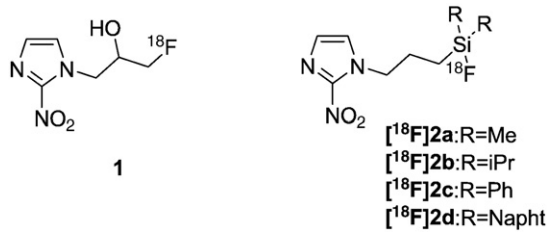

Fig. 1. [^{18}F]-FMISO **1** and its silicon analogues [^{18}F]**2a**, **2b**, **2c**, **2d**.

2. Experimental procedures

2.1. Reagents and instrumentation

Trichloro(chloropropyl)silane, chloro-(3-chloropropyl) dimethylsilane **3a** and 3-(ethoxy-dimethylsilyl)propylamine (**6**) were purchased from Sigma/Aldrich and used without further purification. Unless otherwise stated, reactions were performed under a nitrogen atmosphere using freshly distilled solvents. All reactions were monitored by thin-layer chromatography with Merck silica gel 60 F254 pre-coated aluminum plates (0.25 mm). Flash chromatography was performed with indicated solvents using silica gel (particle size 30–63 μm) purchased from Merck. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance at 300 MHz for ^{1}H and 75 MHz for ^{13}C. Chemical shifts are reported relative to TMS, calibrated with chloroform. Coupling constants J are in hertz and are reported as d (doublet), t (triplet), q (quartet).

General high-performance liquid chromatography (HPLC) conditions: Spectra System P1000Xr and monitoring with ultraviolet (UV) detector Spectra Series UV100; wavelength used: 254 nm. Varian Pursuit XRs 5 C18 (250*4.6 mm); 0.5 or 1 ml.min^{-1}, EtOH/H_2O or MeCN/H_2O: 80/20.